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PIEZOELECTRIC PROBES AND THEIR CAPACITY TO MONITOR TIME VARYING VISCOSITY

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University

By

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Biomedical Engineering
University of Virginia, 2010
Virginia Commonwealth University, 2012

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August 2012

Acknowledgements

I would first like to thank my advisor, Dr. Karla Mossi for all of her support. Despite my limited knowledge in Mechanical Engineering, she did not hesitate in giving me an opportunity to work with Smart Materials and the following project. Her approachable manner and continued patience throughout this journey has been crucial to my success here.

I am grateful to Dr. Kevin Ward for introducing me to such an appealing and promising topic, Dr. Gerald Miller for agreeing to act as my co-advisor, and Dr. Ding-Yu Fei for taking the time to be a part of my committee. Thank you for all of your helpful feedback.

I'd also like to thank my lab mates: Ugur Erturun, for his willingness to get me adjusted in the lab and Dale Farkas, for his expertise and aid in fabricating my prototypes.

And most importantly I'd like to thank my family for their love and support. I have been blessed not only with a supportive immediate family, but an equally encouraging extended family, as well. Thank you for your prayers and faith in my ability to succeed. Thank you to my younger siblings, for making my job as the oldest effortless.

Lastly, the biggest thank you goes out to my incredible parents, for never allowing me to give up and for being my motivation and reason for achieving everything I already have and all that I plan to accomplish. This is for you.

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Abstract

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Real-time, bedside observation of patient clotting is essential in various surgeries in the operating room (OR), but specifically during cardiac surgeries. The objective of this thesis is to design and test a new piezoelectric device that can be used for viscoelasticity measurement with time as a Point of Care (POC) test. Slow turnaround times (TAT) of current methods to monitor blood viscoelastic changes in patients have led to excessive bleeding and the need for blood transfusions in many situations

(Despotis et al, 1997). This study shows that the phase shift produced by a resonator sensor can be related to the viscosity of a liquid. By monitoring a phase shift between an actuator and sensor pair, a numeric relationship can be generated and suffice as a calibration curve for each probe. At a calculated error averaging a maximum of 2%, and coefficient of determination and correlation coefficient exceeding 0.95, two probes have been tested in various glycerin solutions and prepared for whole blood experimentation. They have also been tested in varying temperatures to simulate effectiveness in a dynamic environment, similar to that of clotting whole blood.

CHAPTER 1

A. Objectives

The goal of the following research project is to design, test, and evaluate a piezoelectric to monitor viscoelastic changes with time. The device consists of two probes: an actuator and sensor. The actuator provides displacement through different frequencies, while the sensor monitors wave propagation. A phase shift from actuator to sensor has direct proportionality to viscosity, and ideally blood coagulation changes. Piezoelectric probes have been designed, built, and tested in different concentrations of glycerin and water to simulate blood. By designing and characterizing a piezoelectric composite, it is hoped that its suitability for viscosity-measuring applications can be evaluated.

Three different designs were considered in order to meet various minimum requirements. The probes had to be small in size so as to fit into a standard vacutainer; blood samples are usually stored in 3 mL vacutainers. Using small containers also means that very small volumes of blood are required for testing. For efficiency purposes and the safety of the patient, it is important that less than 2 mL of blood be required. The probes also needed to be easy to dispose of. Durability and repeatability are other crucial factors required of the final prototype. The fragility of the piezoelectric ceramics

made it imperative that the design be durable enough so that they would not break throughout testing, especially with the weight and pull of the relatively heavy wiring at the end of the probe. Different probes should also produce the same results when measuring identical mediums. As stated later in this thesis, reproducibility and durability are the most difficult requirements to fulfill, however, once the probe is manufactured in mass variability will be very limited and many of the differences in structure and results will no longer be significant. Once all of these requirements have been met and capabilities of measuring dynamic viscosity have been demonstrated, the probes will be ready to be used for monitoring viscoelastic changes in time varying fluids.

The layout of this thesis is separated into three chapters. Chapter 1 will introduce piezoelectrics, relevant prior art, the clotting cascade, and other background information necessary for understanding blood coagulation monitoring. In Chapter 2, the materials, experimental setup and overall methodology are discussed. Lastly, Chapter 3 discusses the results of various experiments, concluding remarks, and hypothesizes some challenges and additional future experiments.

B. Background and Literature Review

I. Piezoelectricity

Piezoelectricity comes from the Greek words meaning 'electricity from pressure'. Piezoelectrics are a special class of materials in which a strain results in an electric field inside the material; piezoelectric materials develop an electric potential due to a mechanical stress on the solid. Conversely, they can also deform due to an electric field. Common uses of piezoelectrics include actuators, microrobotics, non-destructive testing, and non-invasive medical diagnostics ("Piezoelectric Ceramics," 2011).

The piezoelectric effect states that tension and compression generate voltages of opposite polarity in proportion to an applied force. This effect is commonly employed in sensing applications, such as force or displacement sensors. The inverse piezoelectric effect is just the opposite of the piezoelectric effect. Here, a voltage-generating crystal exposed to an electric field lengthens or shortens according to polarity of the field, and in proportion to the strength of the field. This is used in actuation applications such as in devices that control positioning and generation of ultrasonic signals ("Piezoelectric Ceramics," 2011).

Relationships between an applied force and the resultant response of a piezoelectric ceramic are dependent upon several factors: the piezoelectric properties of the ceramic, and the size, shape, and the direction of the mechanical and electrical

excitation. Three axes - X, Y, and Z (or 1, 2, and 3) - are used to identify directions in an element. Axis Z is parallel to the direction of polarization in a ceramic (Figure 1).

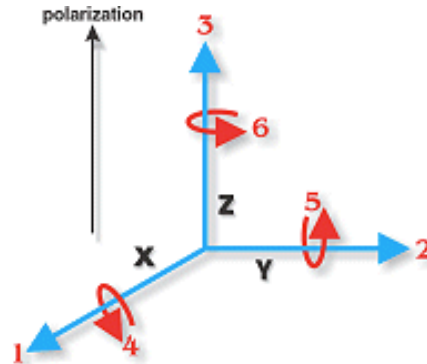


Figure 1. Directions of forces in an element.

There are several piezoelectric constants, such as: the piezoelectric charge constant, piezoelectric voltage constant, permittivity, dielectric dissipation factor, and the frequency constant. The piezoelectric charge constant, d , is the polarization generated per unit of mechanical stress applied or, alternatively, the mechanical strain experienced per unit of electric field applied. The voltage constant, g , is the electric field generated for each unit of mechanical stress applied or, the mechanical strain for each unit of electric displacement applied. Permittivity is also referred to as the dielectric constant, ϵ . This constant is the dielectric displacement per unit electric field. Each constant or coefficient is presented as a variable with double subscripts, representing a ratio in most cases. These subscripts symbolize both the electrical and mechanical values on the orthogonal, three-dimensional axis. The first is associated with voltage

and charge, and the second with stress or strain. For example, g_{33} could signify an electric field in direction 3 per unit stress applied in direction 3, as well.

PZT, or lead zirconate titanate, is one of the most commonly used piezoelectric materials. PZT is generally not used in its natural form, but rather doped with acceptors or donors. Hard PZTs are fixed with electron acceptors, which make it difficult to change the ceramic's properties; they are ideal for more high-powered applications. Soft PZT is fixed with electron donors, making it relatively easy to polarize. This ceramic is normally used in both sensors and actuators, as it is in these experiments. In the table below, there are examples of variations between soft and hard ceramics (Table 1). The last characteristic, 'Polarization/Depolarization,' is the key variation in the use of soft ceramics for the following project.

Table 1. Comparison of Soft Ceramics to Hard Ceramics.

Characteristics of Soft Ceramics and Hard Ceramics Compared

Characteristic	Soft Ceramic	Hard Ceramic
Piezoelectric Constants	larger	smaller
Permittivity	higher	lower
Dielectric Constants	larger	smaller
Dielectric Losses	higher	lower
Electromechanical Coupling Factors	larger	smaller
Electrical Resistance	very high	lower
Mechanical Quality Factors	low	high
Coercive Field	low	higher
Linearity	poor	better
Polarization / Depolarization	easier	more difficult

Piezoelectric sensors are devices that can measure pressure, acceleration, strain, or force by converting vibrations to an electrical charge. A flexional sensor, or bending sensor, measures a force exerted in the direction of polarization of the ceramic element(s) and consequently bends, elongates, or contracts along the plane perpendicular to the direction of the polarization. A typical piezoelectric sensor will only generate a signal when it experiences a change in the applied force or pressure. Bending sensors are utilized as opposed to axial sensors because they have a lower stiffness, and therefore a lower resonance frequency, implying minimal impedance. They also have lower mechanical and electrical impedance, making them compatible with relatively simple amplifiers. Flexional sensors like the one used in the following experiment can be mounted by one free end, called cantilever mounting. The purpose of the cantilever is to act as a vibration source (Wang et al., 1999).

Actuators convert a voltage or other electrical signals into precisely controlled physical displacements that can be used to finely adjust machining tools or lenses in optical equipment. The energy efficiency of an actuator is independent of size, making it advantageous and less of a constraint on piezoelectric motors. Unlike electromagnetic motors, piezoelectric motors do not generate noise.

Smart materials are materials that go through a transformation due to physical interactions, specifically using a feedback system to sense environmental changes. Piezoelectric materials are an example of smart materials. Ceramic elements are made piezoelectric by exposing elements to a strong direct current electric field. Typical applications include almost any piezoelectric application, such as generators, sensors,

actuators, and transducers. Piezoelectric sensors are also ideal in the application of health monitoring of structural integrity. Aging structures, such as space and aircrafts, need to be analyzed for their structural integrity in order to determine the structure's remaining life (Giurgiutiu, 2008). Some limiting factors to the use of ceramics like piezoelectrics are size, fragility, weight and cost.

The following design utilizes PZT, lead zirconate titanate. PZT was discovered in the mid 1900s and has become a prevalent ceramic due to its high piezoelectric and dielectric properties. The main components of PZT are titanium oxide, lead oxide, and zirconium oxide.

Piezoelectric ceramics, like any other body of mass, resonate freely and at higher amplitude at their resonant frequency (f_r). The resonant frequency is where the oscillation amplitude is at its maximum, while the anti-resonant frequency is where the oscillation amplitude is minimal (f_a) (Jordan et al., 2001). Figure 2 shows an ideal impedance curve at a ceramic's resonance frequency.

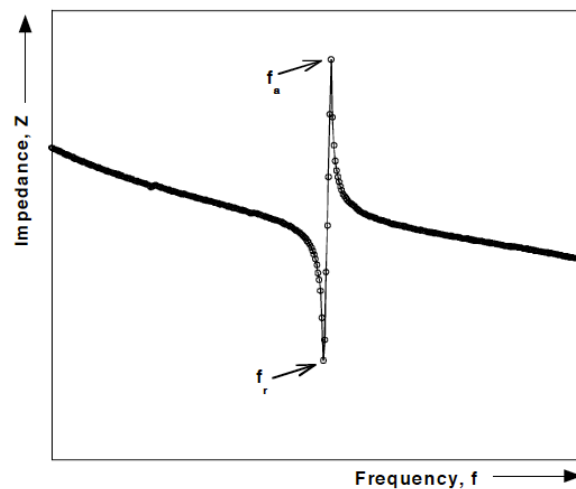


Figure 2. Impedance of Piezoelectric ceramic at its Resonance Frequency (f_r) (Jordan et al., 2001).

II. Resonance Sensors

Resonator sensors use vibration to detect changes in frequency and phase. They are used for various applications including sensing liquids and gases, viscosity, force and mass, and temperature. In the following experiments, resonance sensors were used to measure viscosity. A mechanical resonator is immersed in a moving fluid and the momentum of the vibration is transferred from one part of the resonator to another by the fluid flow, leading to differences in phase of the resonator motion between one point and another (Landon, 1985). When a mechanical resonator is surrounded by liquid, the decay time of the resonator can easily be affected. The size of the sensor is another significant variable. Compact sensors tend to operate at high frequency levels. However, at high frequency levels greater than 20 KHz, the penetration depth of the vibratory disturbance into the liquid is extremely small, providing less accurate data. In water, for example, the penetration depth is only 4 μm at a frequency of 20 KHz. Also essential in determining the reliability of viscosity measurements are the condition and cleanliness of the probe surfaces (Landon, 1985). There is a wide range of applications of resonator sensors, such as in the monitoring the structural health of aging buildings and aircrafts (Giurgiutiu, 2008).

If the liquid in use is Newtonian, the time constant can be related to the dynamic viscosity by the Stokes theory, which describes the motion of a fluid. However, whole blood is non-Newtonian. Due to its non-Newtonian state and extreme sensitivity to the surrounding environment, whole blood is almost impossible to test accurately. This limitation is illustrated in the lack of *in vivo* capable methods.

III. Coagulation and the Blood Cascade

The end product of this research will ideally result in a probe that rapidly monitors the coagulation process of any given patient. It is therefore crucial that the cascade and sequence of clotting be studied and understood to ensure accurate results.

The blood coagulation process is an important part of homeostasis. The clotting cascade is made up of an intrinsic pathway, extrinsic pathway, and a final common pathway.

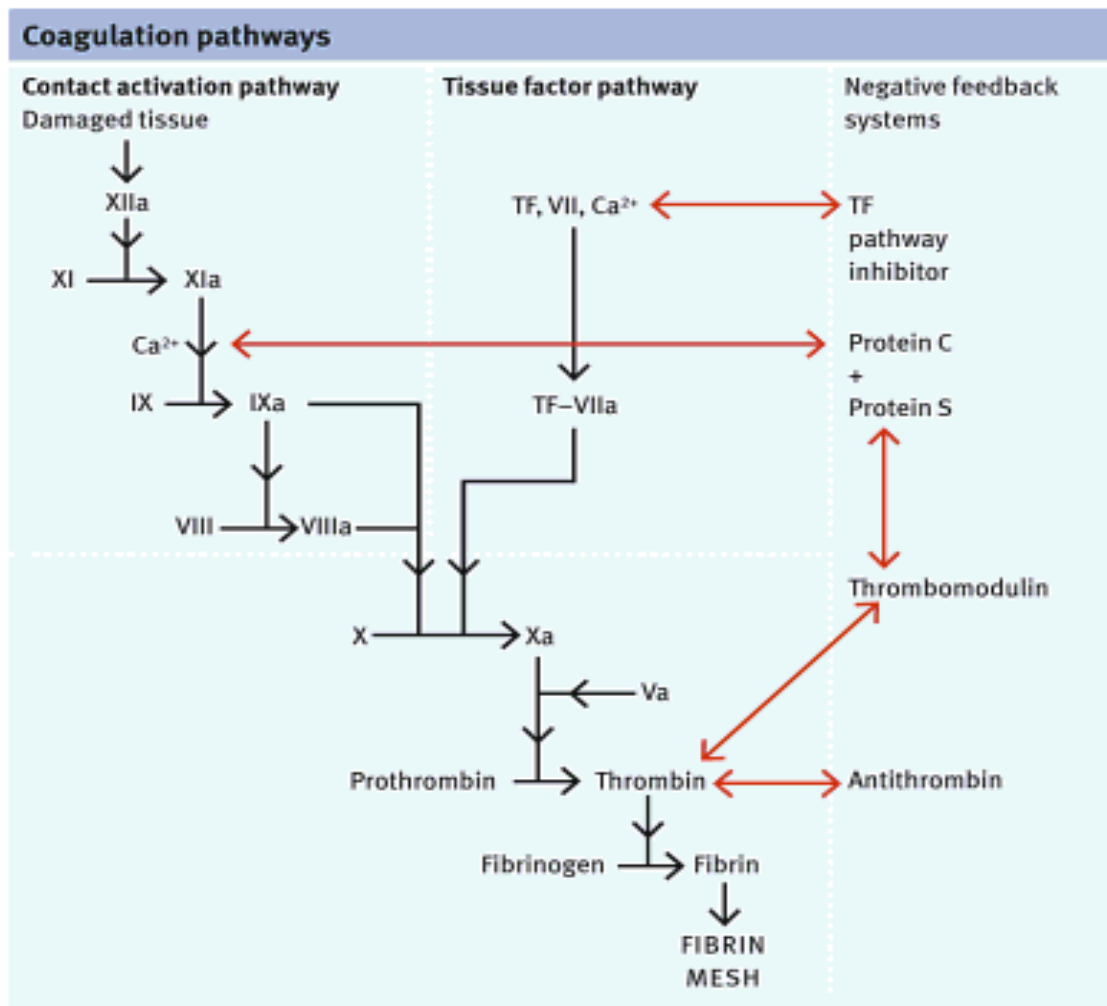


Figure 3. Coagulation Pathways (Lip et al., 2010).

Figure 3, outlines the two pathways that merge near the end of the coagulation process. The intrinsic pathway, or contact activation pathway, is initiated by trauma to blood or exposure of blood to exposed extracellular matrix molecules in a damaged vessel wall. It begins with adsorption of the contact protein factor XII. A negatively charged surface is required to initiate the intrinsic pathway. The extrinsic pathway, or tissue factor pathway, is initiated by the release of tissue factor (TF) upon vascular injury. TF (or factor III) binds with Factor VII on the surface of a phospholipid membrane. The common pathway produces fibrin and, more importantly, a clot. The intrinsic and extrinsic pathways converge at the activation of factor X to Xa. Factor Xa hydrolyzes and activates prothrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Ultimately the role of thrombin is to convert fibrinogen to fibrin and to activate factor XIII to XIIIa.

During any surgery, the coagulation and inflammatory processes are disturbed due to hemorrhage and blood transfusion, as well as surgical stresses. The patient's circulating blood quickly reacts to a disruption of vascular endothelium in order to limit the bleeding. Initial hemostatic response is triggered by tissue factor (TF). Circulating platelets contribute to localized thrombus formation at the site of vascular injury first by adherence to vWF, or the von Willebrand factor. VWF is an important glycoprotein involved in hemostasis. A deficiency of vWF can lead to several different blood diseases including von Willebrand disease (vWD).

IV. Prior Art

Monitoring the blood coagulation process is critical during anesthesia and surgical procedures. Conventional coagulation monitoring methods such as Point-of-Care (POC) analyzers have several drawbacks, including requiring specialized laboratory personnel, large samples of blood, and analysis of only one sample at a time. It is crucial that turnaround time (TAT) between sampling and coagulation testing be reduced, therefore utilizing near patient testing (NPT) and more effective POC analyzers. TAT is the time interval between physician ordering and therapeutic intervention, and is an important assessment of POCs (Curry et al., 2007). In the following paragraphs, various monitoring methods will be introduced and compared.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are standard tests to monitor functioning of the coagulation system and the effectiveness of anticoagulation therapy. Central laboratory testing of these parameters can take up to one hour (Hirsch et al., 2001). PT and aPTT are the most common *in vitro* screening tests for coagulation abnormalities. aPTT is used clinically for monitoring of different anticoagulants, with a specific calibration required for each. Both PT and aPTT do not provide information on *in vivo* interaction of platelets and coagulation factors, nor do they estimate stability of hemostatic thrombus because both tests terminate before fibrin is polymerized. Platelet function assays, thrombelastography/metry, and calibrated thrombin generation assays are all examples of specialized coagulation assays. Point of Care monitoring of coagulation will become more important in the

future because hemostatic interventions are increasingly available, as described in a later section (Tanaka et al., 2001).

Activated clotting time (ACT) was the first system used to monitor coagulation during a cardiopulmonary bypass, or CPB. ACT uses an activator to speed up the clotting process via the contact pathway of the blood cascade. The most commonly used activators are kaolin and celite. Some disadvantages of ACT include the inability for ACT devices to consider duplicate patient samples, lack of standardization for comparison between labs, and inadequate quality control (QC) methods. Another variable is the use of different assays or devices for measuring ACT. All of these factors greatly limit the ability to compare ACT results (Prisco et al., 2008).

A few of the current POC analyzers overcome some of the limitations of routine laboratory coagulation tests such as ACT. Such tests include the Thrombelastograph (TEG), rotation Thromboelastometry (ROTEM), and Sonoclot Analyzer. Each one of these devices assesses viscoelastic properties of blood (Ganter et al., 2008).

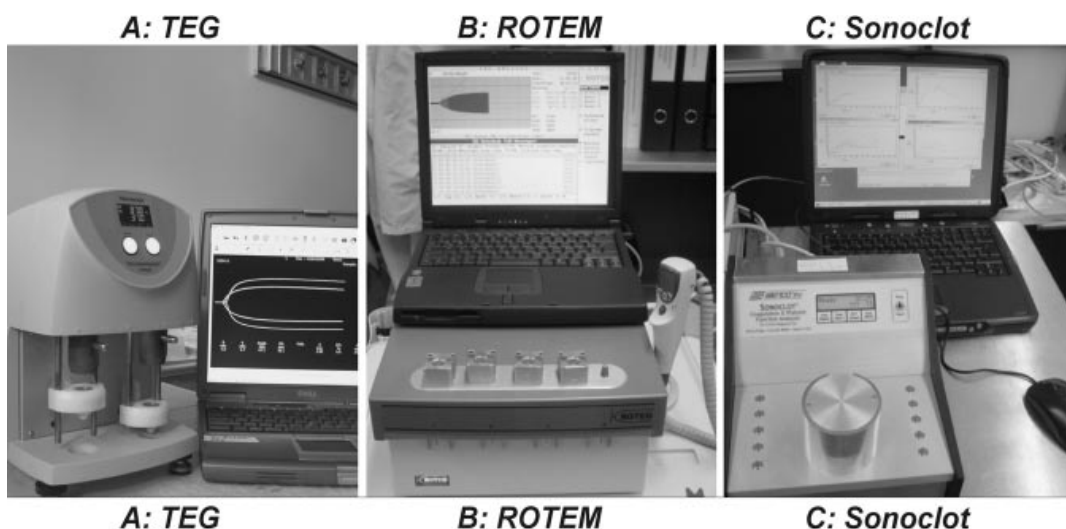


Figure 4. Prior Art Examples: Images of Viscoelastic POC Devices (Ganter et al., 2008).

The TEG consists of a pin and cup, or inner and outer cylinder, respectively. Small volumes of whole blood samples are placed inside the cup and rotated back and forth, at a temperature of 37° C, or body temperature. The movement of the pin represents displacement and is plotted on a graph, or thromboelastogram. A torsion wire suspends the pin to the cup, which is coupled to a recorder or monitor during the process (Figure 5a). Torque is transmitted through the wire and to the monitor as the blood starts to clot and increases with the strength of the clot. Inversely, it decreases with lysing, or breaking, of the clot. Reaction, or lag, time before the pin starts to move defines the time necessary to form a clot or fibrin network (Evans et al, 2008b).

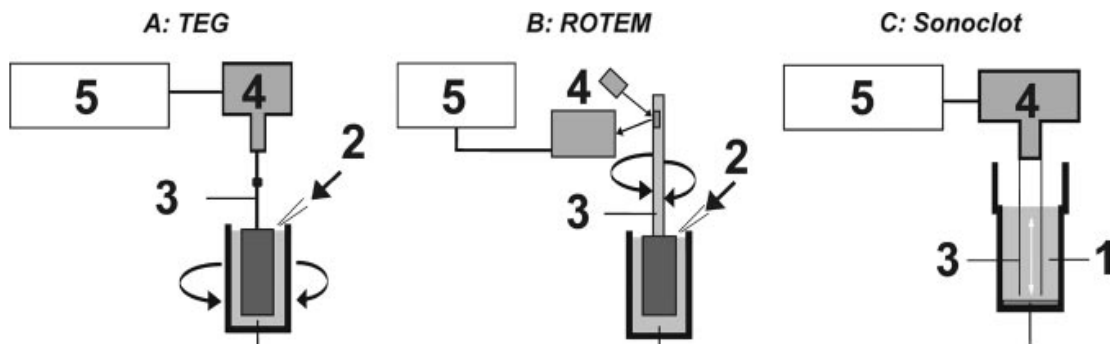


Figure 5. Step-by-step Schematic of Viscoelastic Point-of-Care Coagulation Devices (Ganter et al., 2008).
A: Rotating cup with sample (1), coagulation activator (2), pin and torsion wire (3), electromechanical transducer (4), data processing (5).
B: Cuvette with blood (1), activator added by pipetting (2), pin and rotating axis (3), electromechanical signal detection via light source and mirror mounted on axis (4), data processing (5).
C: Blood sample in cuvette (1) containing activator (2), disposable plastic probe (3) oscillating in blood sample mounted on electromechanical transducer head (4), data processing (5).

The TEG has been used bedside to monitor hemostasis during cardiac and hepatic surgeries. Using analytical software, it provides coagulation information through a graphical representation of the process of clot formation and lysing. The parameters

measured are: an alpha angle, reaction time (R), clot formation time (k), amplitude at an hour, and the maximum amplitude. These five parameters reveal information about clotting factors, platelet function, and other coagulation related factors (Figure 6a).

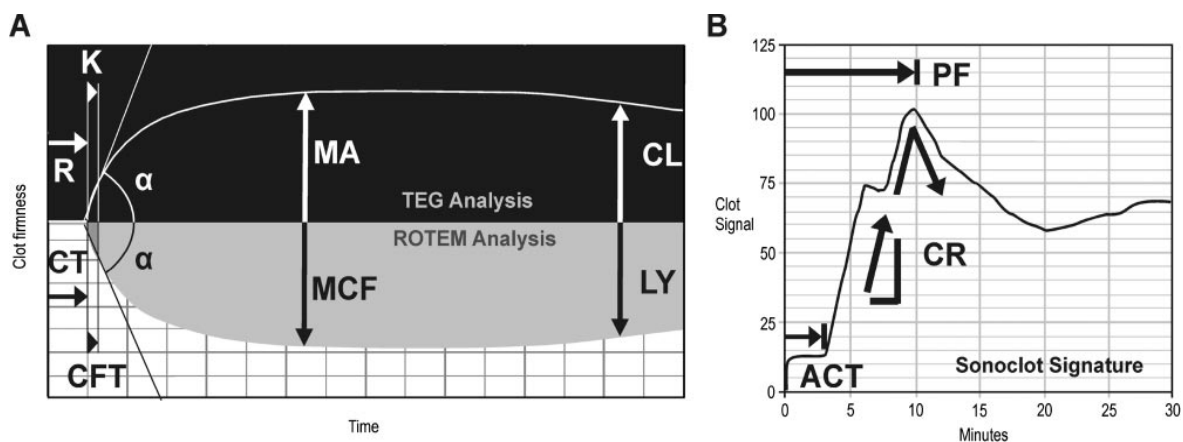


Figure 6. Graphical Representation of the TEG, ROTEM, and Sonoclot (Ganter et al., 2008).

A) Top: Thrombelastograph tracing: R= reaction time; K= kinetics; Alpha=slope between r and k; MA=maximum amplitude; CL= clot lysis. Bottom- ROTEM tracing: CT= clotting time; CFT= clot formation time; Alpha=slope of tangent at 2 mm amplitude; MCF= maximal clot firmness; LY= Lysis.

B) Sonoclot Signature: ACT=activated clotting time; CR=clot rate; PF= platelet function.

A major drawback of the TEG is that the shear force applied substantially weakens the clot. It is also unable to detect the establishment of the initial clot in whole blood samples (Evans et al., 2008b). The ROTEM, or Rotation Thromboelastometry, uses similar technology to the TEG, where rather than a torsion wire, the rotation is initiated by an optical detection system and the inner cylinder, or pin (Figure 5b).

The Sonoclot Analyzer, also known as the Sonoclot Coagulation and Platelet Function, was introduced in the 1970s. Its measurements are also based on detecting viscoelastic changes in a sample. The setup involves the use of a plastic probe,

transducer, and cuvette of the sample. The probe is inserted into the activated, or inhibited, blood sample and oscillates (Figure 5c). Impedance changes occur as the clot develops and are measured in order to produce a graph called the Sonoclot Signature and quantitative results. The activated clotting time (ACT), the clot rate (CR) and the platelet function (PF) are each provided (Figure 6b). While the TEG/ROTEM's output has a direct relationship with the strength of the clot, the Sonoclot's output is sensitive to the changes in viscosity throughout the clotting process. A disadvantage of the Sonoclot is in the use of heparin. In heparinized samples platelet activation is inhibited, making it difficult to assess platelet function (Tucci et al., 2006). Similarly, it is not effective in detecting aspirin therapy in patients. Aspirin slows clotting by inhibiting platelet activation (Hett et al., 1995). The Sonoclot also does not directly measure thrombin formation (Tanaka et al., 2004).

V. Viscosity

Viscosity is a material property defined as the ratio of shear stress to rate. In Newtonian fluids, the ratio is 1.0. Water and most gases are Newtonian fluids. Whole blood, however, is non-Newtonian and therefore a constant coefficient of viscosity cannot be defined. The viscosity of blood varies according to several factors including temperature, hematocrit, properties of red blood cells, velocity within vessels, and size of the vessel. These factors make it more feasible to measure blood viscosity *in vitro*, as seen with major coagulation monitors. Viscosity is measured in Poise (P) or Pascal-seconds (Pa-s) and normally ranges between 1 and 4 cP in whole blood, at body

temperature (Evans et al., 2008a). Plasma viscosity is about 1.5 cP, but 3.2 cP in whole blood, assuming it has an average hematocrit of 40-45%. Several existing methods for measuring blood coagulation using vibrating sensors have been designed and tested but never fully implemented, such as various quartz crystal sensors (Westerhof et al., 2010).

Rheometers are used to measure fluids with varying flow conditions, because viscometers can only analyze fluids at a constant flow rate. Rheometers tend to measure liquids that lack a single value of viscosity, such as whole blood. It is almost impossible to measure blood *in vivo*, due to the changes in flow rates, different vessel sizes, varying body temperature, and several other factors. This has led to a considerable amount of focus on *in vitro* methods (Bodner et al., 1997).

Red blood cells are the main difference between whole blood and plasma, making hematocrit, or red blood cell percentage, the key to viscosity differences. One commonly used formula relating viscosity to hematocrit is:

$$\text{Equation 1: } N = N_{\text{plasma}} * (1 + 2.5Ht)$$

In equation 1, the variable N represents viscosity (in cP) and Ht is hematocrit, in decimal form. The graphs below explain the derivation of the equation (Figure 7).

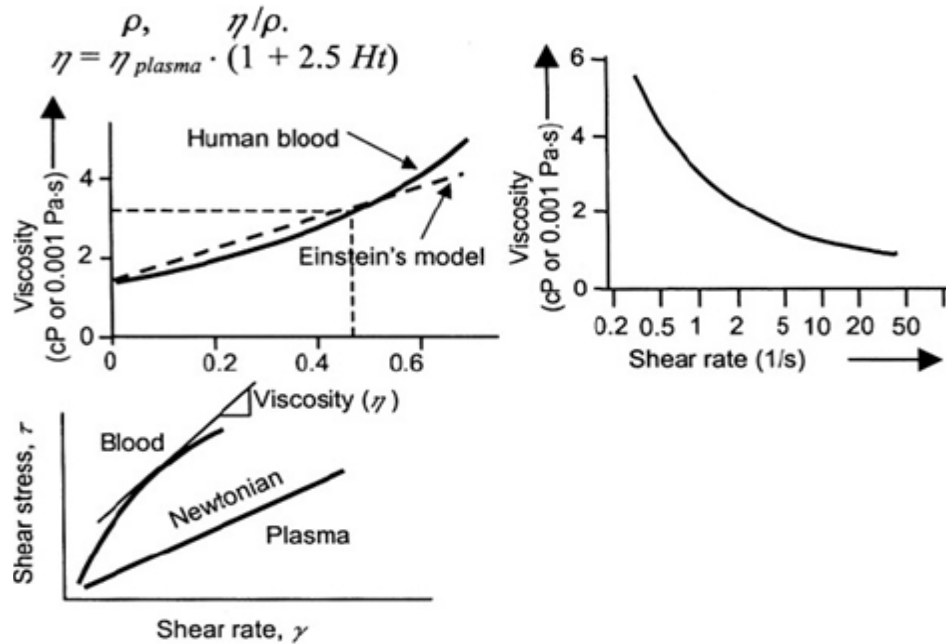


Figure 7. A) Viscosity as a function of hematocrit. B) As a function of shear rate at a constant hematocrit of 48%. C) Plasma vs. Blood viscosity (Westerhof et al., 2010).

VI. POC Testing

The key objective of Point of Care testing is to generate a rapid result so that appropriate treatment can be implemented, leading to an improved clinical or economic outcome (Cheng et al., 1998). POC testing is sometimes called decentralized testing, near-patient testing, bedside diagnostic testing, or ancillary site testing (Price, 2001). Two types of POC technology have been categorized: small bench top analyzers (blood, gas, and electrolyte systems) and hand held, single use devices (urine albumin and blood glucose). The bench top systems are smaller versions of laboratory analyzers in which vulnerable operator dependent steps have been automated—like automatic flushing of a sample after analysis, calibration, and quality control. Hand held devices

have been developed using microfabrication techniques. They are simple enough to handle and use, but internally complex devices. The following probe would most likely be a piece to a bench top point of care system.

The goal of Point of Care technology is to provide reliable, accurate, and cost-effective information about a patient in a brief period of time, ideally less than five minutes. Turnaround time, or TAT, is the most effectively used measurement of a successful POC test. It has been shown that the clinical usefulness of blood tests is dependent on the time between blood sampling and availability of the results.

Advantages of POC testing mainly lie in situations in which there are limited resources or access to facilities. This makes POC the go-to method for innovations catering to developing nations. Point of Care testing is also a promising step for designing for the developing world. With POC testing, patients in rural areas far from health facilities can be conveniently tested for conditions such as high blood glucose levels using a simple kit and a small amount of patient's blood or saliva (Altieri and Camarca, 2001). Resources in such locations are limited, making the idea of a bedside coagulation monitor essential, specifically when it comes to the cost of training and employing personnel that can be saved.

CHAPTER 2

Materials and Methods

A. Probes

In order to design a probe that can be used for monitoring blood coagulation the following constraints were utilized: size, sample volume, wiring, durability, disposability, and reproducibility of results. The probes had to be small in size so as to fit into a standard 3 mL vacutainer. It is important that only a small volume of blood is required for testing (less than 2 mL). The probes must also be durable and provide repeatable data. The vulnerability of the piezoelectric ceramics made it essential that the design be durable enough so that they would not break throughout testing, especially with the weight and pull of the relatively heavy wiring at the end of the probe. Different probes should also produce the same results when measuring identical mediums. The three different prototype designs and their successes are discussed in the following section.

I. First Prototype

At least three different forms of the current probe were tested before selecting a viable prototype. The first probe simply used PZT unimorphs, which was quite unsuccessful due to the obvious fragility of the ceramic. Figure 8 shows a schematic, with the bare PZT labeled. This prototype was consistently breaking, causing us to consider a new, more structured set up.

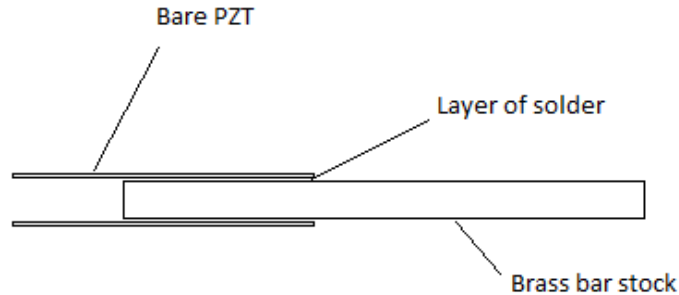


Figure 8. Prototype 1.

II. Second Prototype

The second prototype was that of a pre-stressed ceramic, manufactured by Face[®] International (Figure 9). The pre-stressed PZT's were assembled of multiple layers of the actual piezoelectric material, adhesive, stainless steel, and aluminum; they tended to break apart at various stages, making them unsuccessful, as well. This form of peeling and separation of the layers is called delamination and is a common failure mechanism of composite materials. It was also costly to have these probes

manufactured professionally and sent to the lab for experimentation. A third and final prototype was built in order to overcome the delamination issue of this prototype.

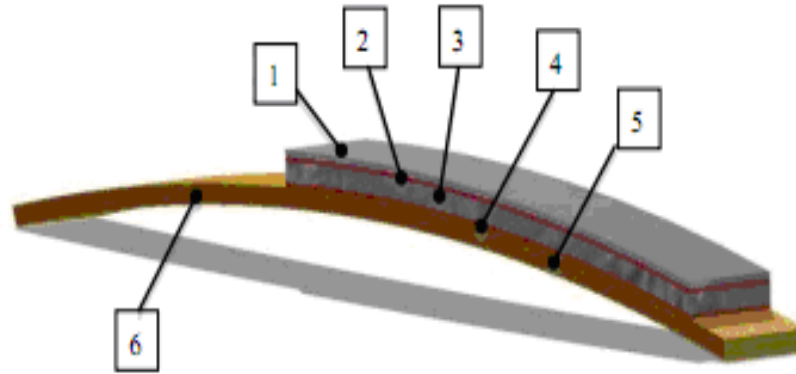


Figure 9. Prototype 2. (1) Aluminum, (2) Adhesive, (3) PZT, (4) Adhesive, (5) Brass, (6) Tab Holder.

III. Third Prototype

The third and final attempt creates more stability and less weight for the fragile ceramic, overcoming the problems of both the first and second prototypes. At this level of experimentation all of the probes were fabricated in house. Individual probes were built to be as identical to each other as possible. Required materials include PZT, copper sheets, hollow brass bars, heat shrink-wrap, soldering material, epoxy glue, and insulated wires.

The probe is made up of two PZT unimorphs, built out of identical piezoelectric polycrystalline ceramics. A unimorph is a cantilever that consists of one active piezoelectric layer and an inactive layer. One unimorph functions as the actuator

(output), and the other as a sensor (input). The actuator vibrates as a function of the application of the current. These vibrations are then transmitted across the approximately 0.5 cm gap to the second probe, or sensor. The vibrations are transmitted through the medium, or glycerin solution. As these vibrations reach the sensor they cause it to flex, as stated by the Piezoelectric Theory. A sheet of ceramic was cut into pairs of 2.3x0.5 cm pieces. Due to the fragility of the thin ceramics, as seen in the first prototype, copper backings (2.5x.6cm) were glued to each piece to maintain structure and attach the unimorphs to the rest of the probe. A coated brass bar then holds them parallel to each other. The square and hollow brass bar is 7x0.5x0.05 centimeters; it was coated with two layers of polyurethane in order to prevent unnecessary electrical conduction during testing. Lastly, two pairs of wires were soldered down to the PZT. Two, approximately 40 cm 22-gauge, solid wires and a short flat unshielded wire were used for each side of the probe. The unshielded wire is used as a connector for the ground wire, to minimize clutter and avoid conduction issues near the soldered point in the middle of the probe. Heat shrink-wrap was also used to help avoid the force of pulling during handling from breaking the ceramic. Figure 10 and 11 display a typical probe of the third prototype. The rubber stopper of a vacutainer was punctured through the middle, allowing the probe in its entirety to fit through the rubber stopper of the vacutainer (Figure 12).

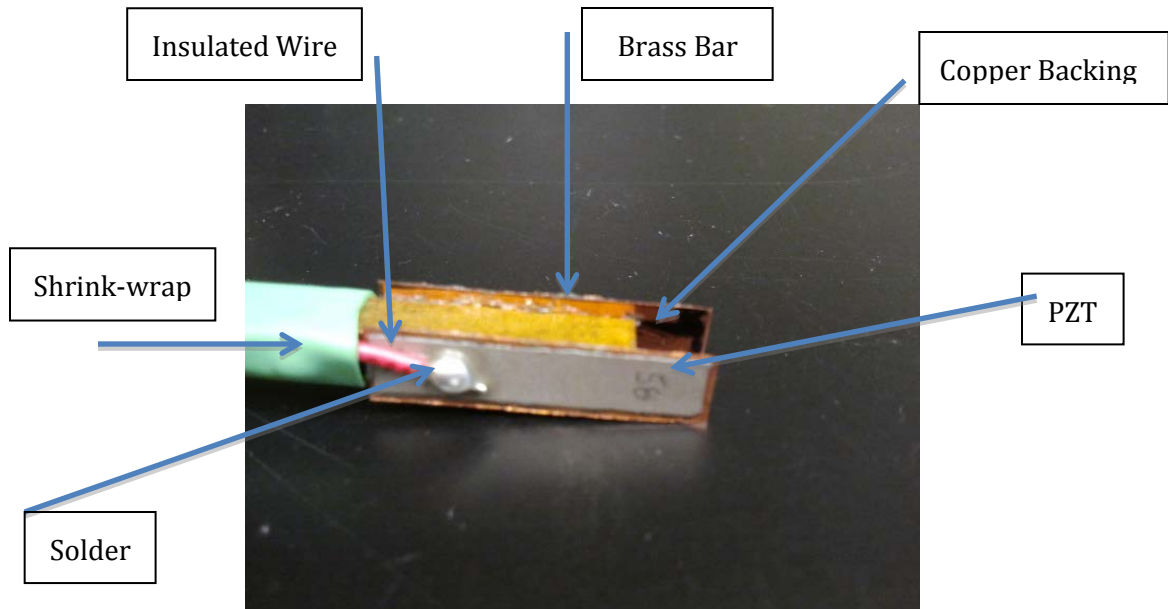


Figure 10. Prototype 3: Typical Probe. Ceramic, copper backing, brass bar, soldered wire, and shrink-wrap visible in photo.

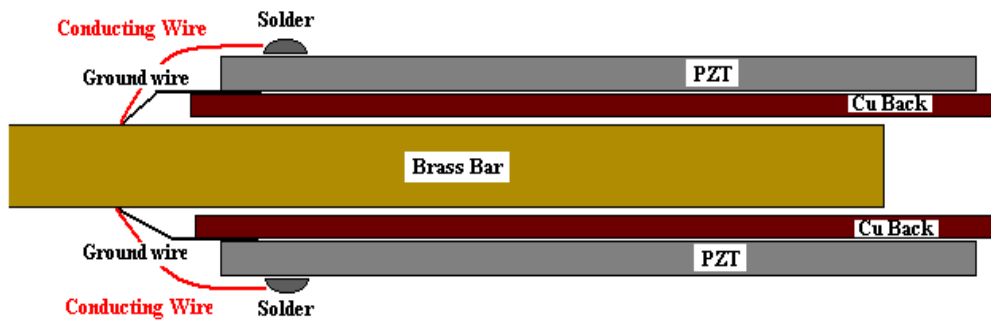


Figure 11. A schematic of probe pieces in their respective order and alignment.



Figure 12. Final Prototype: Photograph of 3 inch probe with vacutainer top.

B. Experimental Setup

In the following experiments, glycerin solutions were used to simulate the sequential process of blood coagulation. Glycerin is commonly used to simulate whole blood due to the ease of manipulating its viscosity, either through temperature change or solution composition (Price et al., 2004). Various glycerin levels were prepared by mixing some ratio of distilled water to glycerin, for ten different solution percentages. For example, a higher percentage of glycerin would correspond to the most viscous form of blood, or a fibrin clot. The following chart is a table of the ten percentages used and their respective viscosities, at room temperature (Table 2). These values were interpolated for the average room temperature of 25 °C, which was not provided in the 1990 "Glycerine- A Review" article.

Table 2. Glycerin Viscosities of Various Glycerin Solution Percentages.

Viscosity (cP)	Solution (%)
0.90	0
1.17	10
1.95	25
2.19	30
3.22	40
5.11	50
9.00	60
28.35	75
164	90
1011	100

A large volume (roughly 20 mL) was mixed and stored in a 50 mL Corning centrifuge tube, in order to eliminate variability of prepared solutions for each trial. Approximately 1.5 mL of solution was poured into a standard 3 mL plastic vacutainer (Figure 13). This approximate level was an appropriate height for the immersion of the pair of unimorphs.



Figure 13. 3mL Vacutainers of Glycerin Solution.

Testing varying levels of glycerin (0-100%) presented several variables and constraints that needed to be kept constant. Most important was the method of holding the solution still while being tested. This involved a clamp that was kept at the same height and pressure for each of the ten solution levels. The vacutainer was originally tested with the clamp at three different levels: below the probe and in the solution, at the meniscus of the liquid, and just below the rubber top. The strongest repeatability was perceived with the clamp right below the probe and along the solution. It was also

crucial that the same volume of solution was consistently used, so a syringe was used for precise measurements. All testing was done in a box lined with a makeshift faraday cage, or aluminum foil to minimize noise from the outside environment (Figure 14). A faraday cage is particularly used in experiments that deal with low currents or high frequencies, or both ("The Faraday Cage," 2010). When more precise measurements are required, it is essential that a cage be used to reduce outside noise.

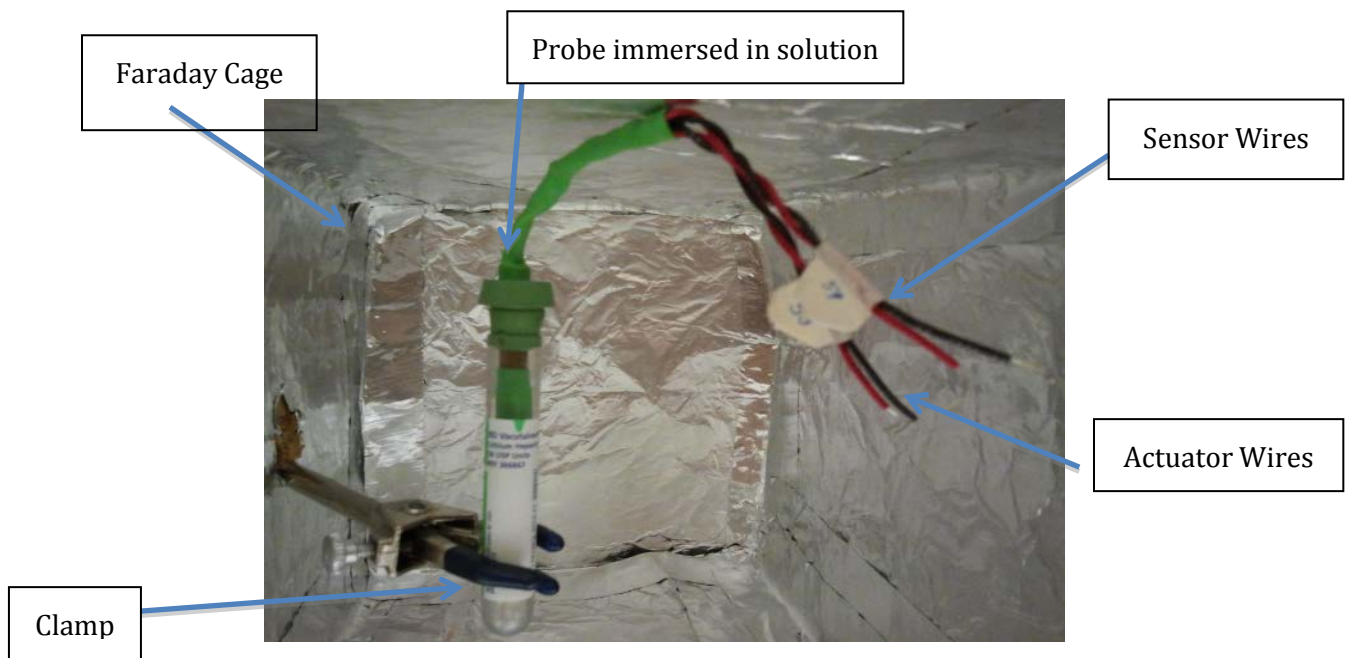


Figure 14. Experimental setup including: the clamp, probe in solution, and faraday cage.

A lab view program was written in order to test for different parameters including capacitance, impedance, gain, and phase (Figure 15). This program was used in conjunction with an impedance analyzer- Hewlett Packard 4194A Impedance/Gain-Phase Analyzer (Figure 16). This impedance analyzer is capable of measuring

impedance between 100 Hz and 40 MHz, and gain-phase in the range of 10 Hz -100 MHz.

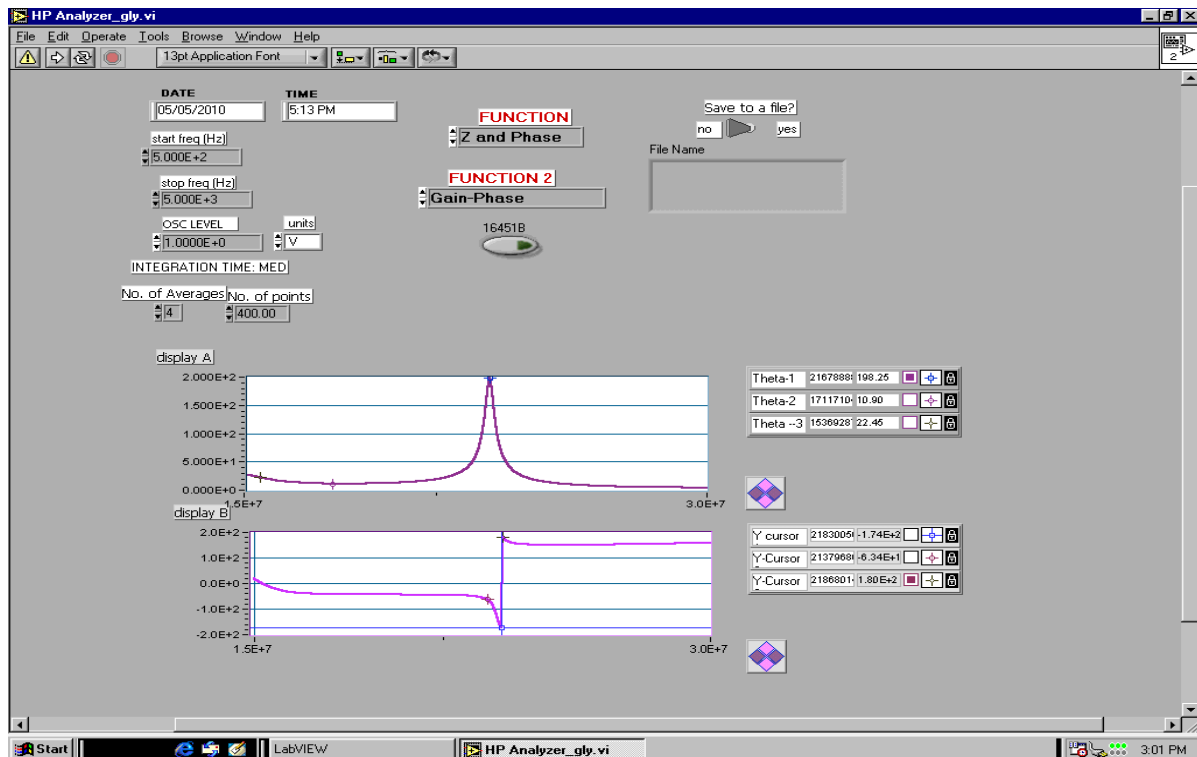


Figure 15. User Interface of Gain-Phase Testing.



Figure 16. Hewlett Packard 4194A Impedance/Gain-Phase Analyzer.

Before selecting constant variables during experimentation, several ranges and values were attempted until consistent results were produced with certain options. A specific range of frequency, number of averages, integration speed, and oscillation voltage were finally chosen for each sample (Table 3).

Table 3. Table of set parameters during testing.

Gain-Phase Frequency Range	500Hz-5 KHz
Impedance Frequency Range	20MHz-40MHz
Oscillation Level	0.5 V
Averaging Time	16
Integration Speed	Medium
Environment Temperature	22-26° C

Once data was collected, it was analyzed using the program Sigma Plot. This statistical program makes it relatively simple to graph large data files. We define a phase shift as the change from a negative to positive phase; since this is best visualized as a peak, calculating the first derivative of the produced graph is beneficial to analyzing the data. The following four figures will display this conversion for both Probe 1 and Probe 2. Figure 17 is an example of a phase shift produced by the impedance analyzer and Lab View program, while figure 18 displays the derived peak produced by calculations from Sigma Plot, for Probe 1. Figure 19 and 20 do the same for Probe 2. Peak magnitudes are irrelevant, and only the locations of the peak were of importance.

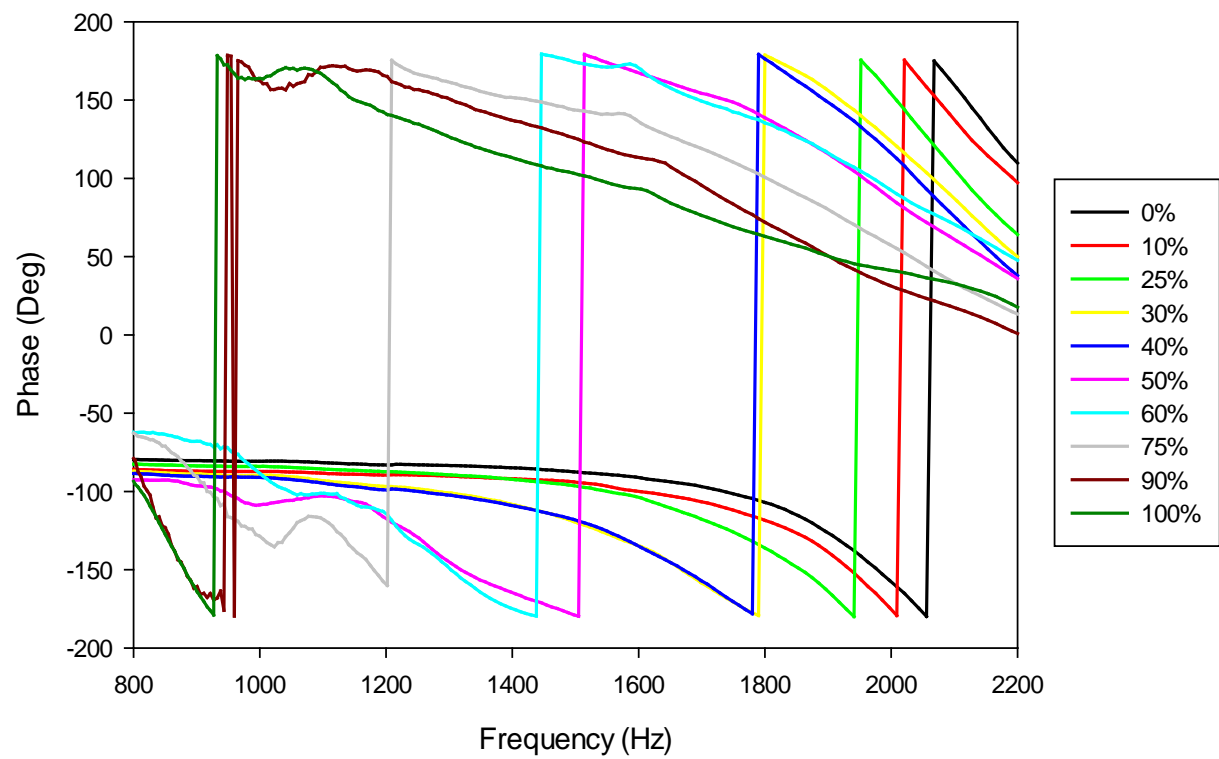


Figure 17. Probe 1 Phase Shifts for all ten glycerin levels.

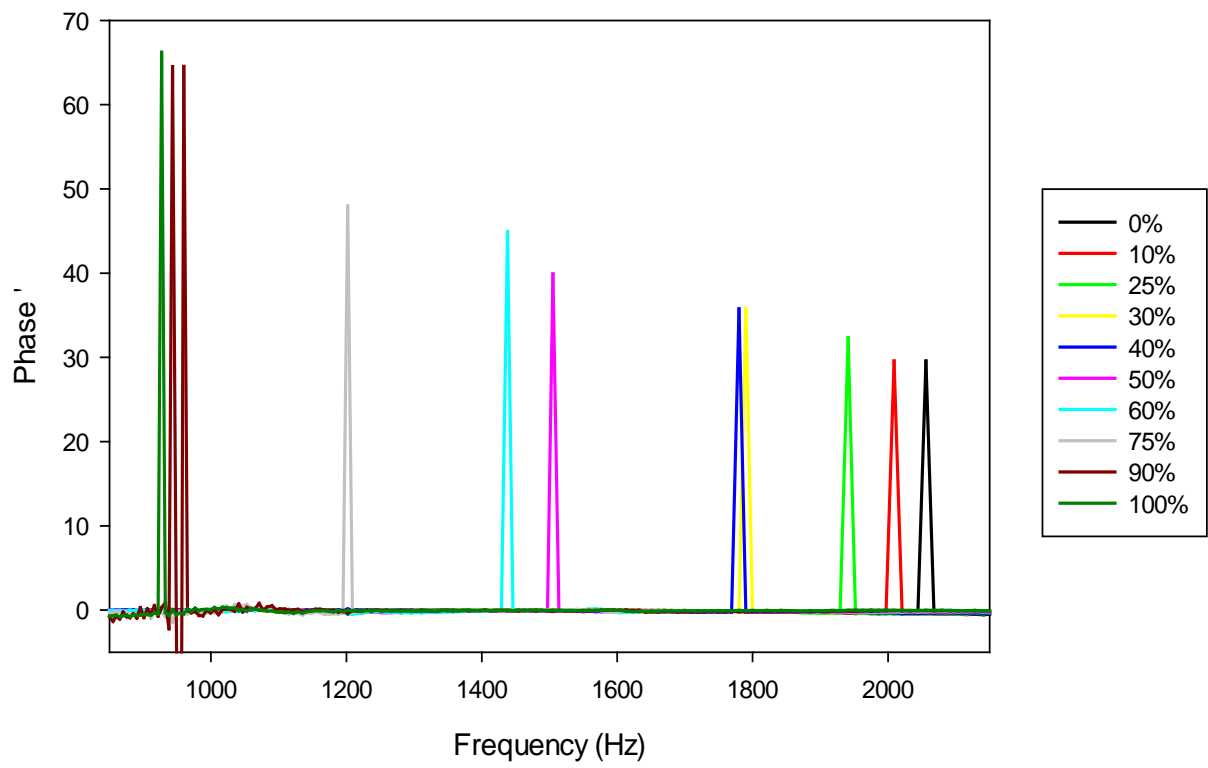


Figure 18. Computed derivative of the phase output of Figure 17.

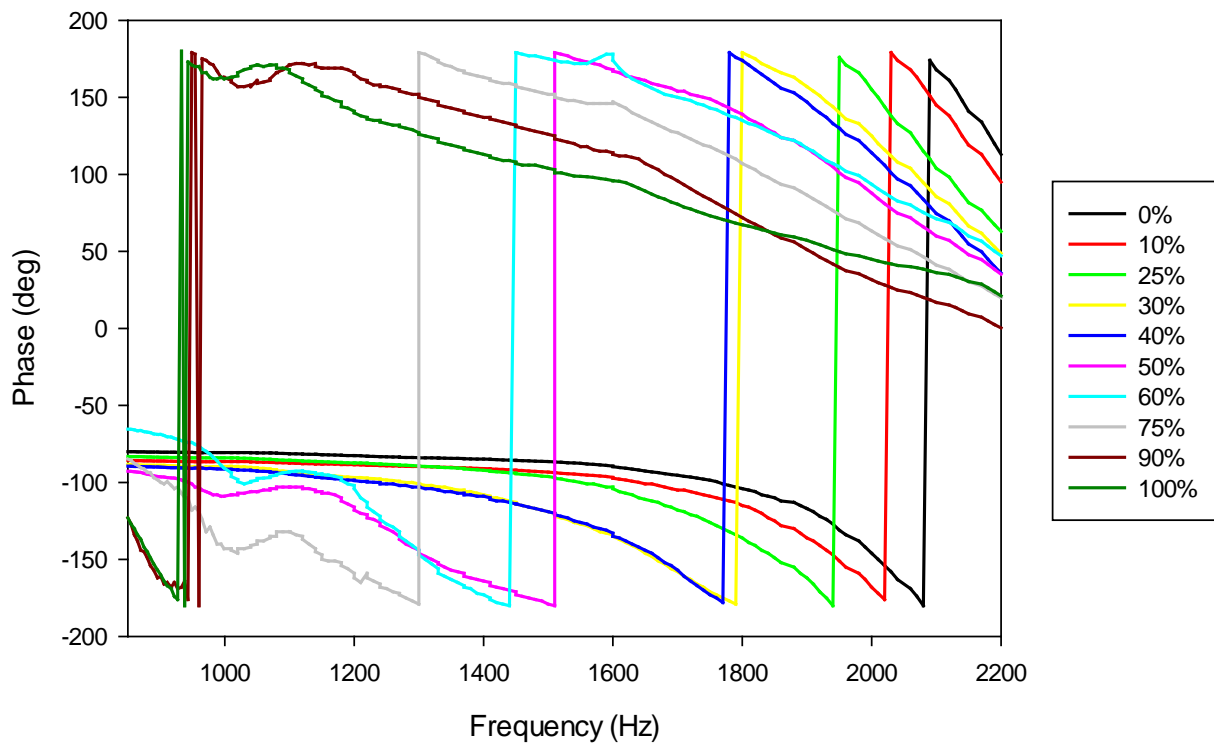


Figure 19. Probe 2 Phase Shifts for all ten glycerin levels.

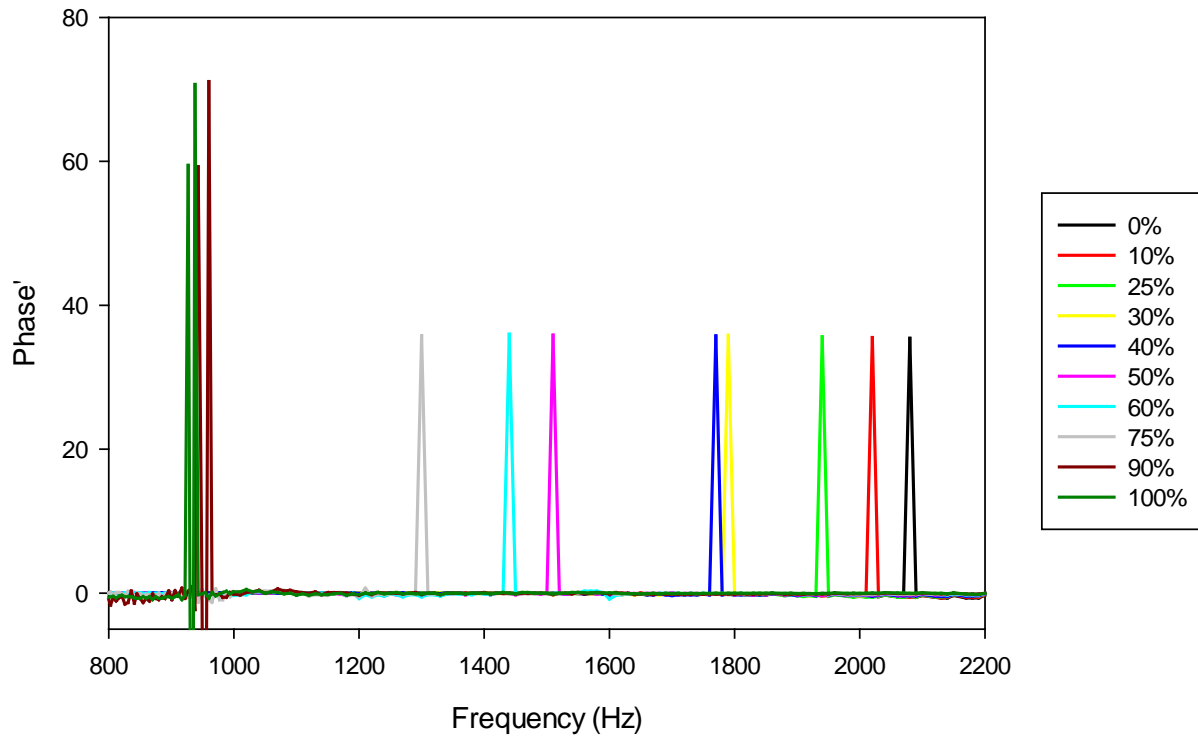


Figure 20. Computed derivative of the phase output of Figure 19.

Since the probes were fabricated manually in house, there is room for significant variability between different probes. Quality assurance tests were crucial in determining prototype usability. In order to test each probe for functionality, both capacitance and impedance were periodically tested as probe properties. Capacitance measurements are generally carried out at 1 KHz and at a low excitation voltage, or in the mV range.

Figure 21, demonstrates a typical capacitance curve of a ceramic unimorph.

Capacitance was tested along various steps throughout the building process. A low capacitance could signify a crack in the ceramic or circuit short. High impedance values combined with consistent capacitance results indicate that the probes are suitable as

either actuators or sensors since the curves are typical for piezoelectric materials. Based on the size, dimensions, and type of ceramic, the capacitance was expected to be within the range of 9 nF to 12 nF.

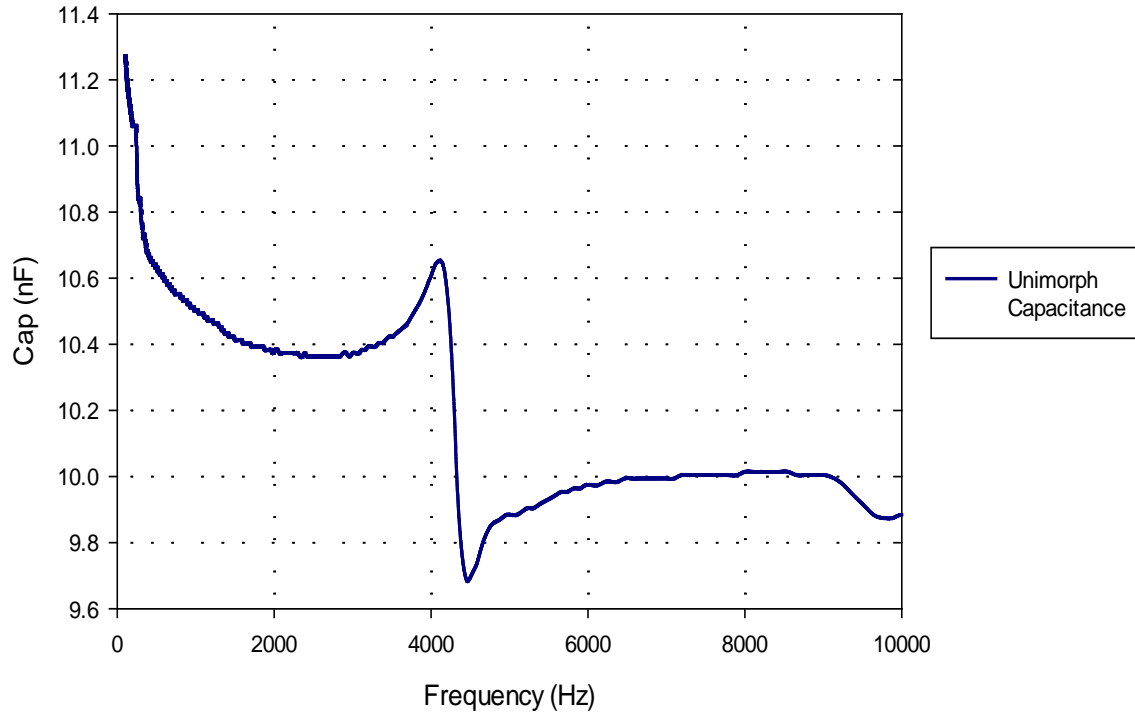


Figure 21. A Capacitance curve for a typical unimorph. At 1 KHz, the capacitance is 10.5 nF.

As for impedance, it was measured before each set of Gain-Phase tests to ensure quality of the probe (Figure 22). Because impedance is a property dependent on environmental variables, measurements were made only in air before experimentation. The actuator is the output and therefore of slightly more importance of the two ceramics, so it was crucial that the impedance of the actuator be consistent for

repeated measurements. An average of an impedance peak at 31 MHz was measured for each actuator.

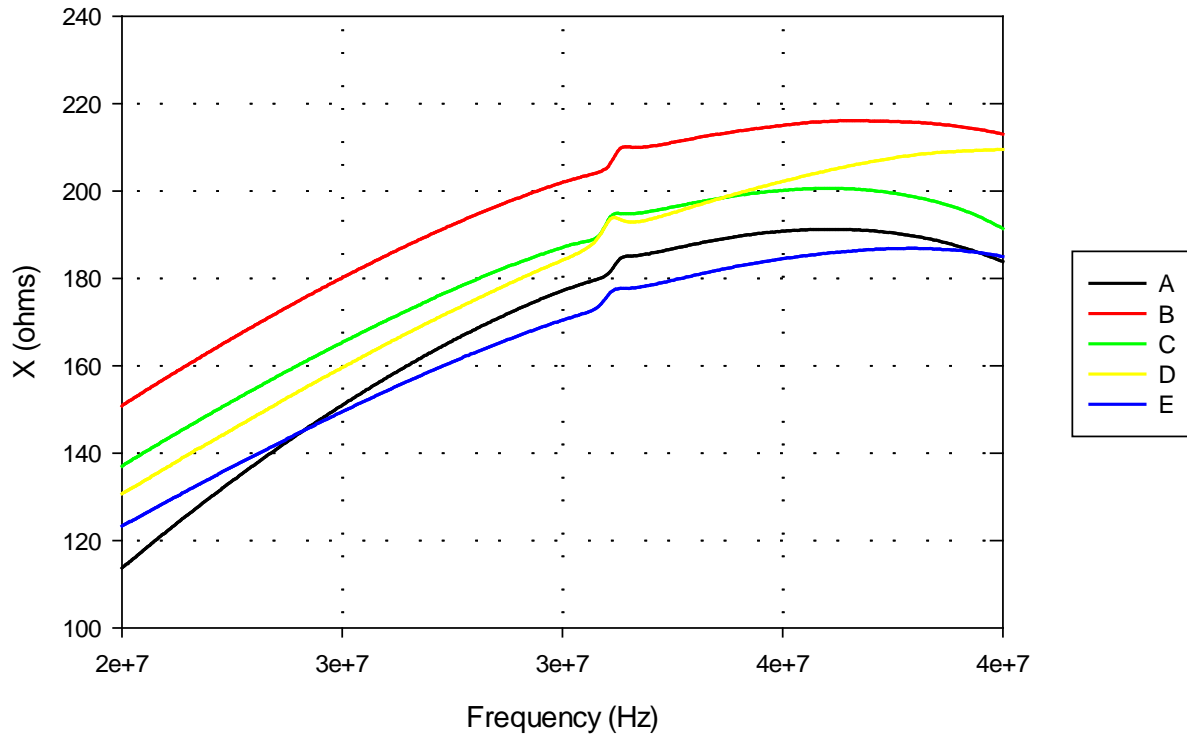


Figure 22. Impedance curves of various probes.

A typical set of experiments consists of five steps. The following steps summarize the order of the different tests done on a standard probe:

- 1- Measure the capacitance of both the actuator and sensor, using a multimeter.
- 2- Test the impedance of the actuator in air (an empty vacutainer) at a range of 20-40 MHz
- 3- Prepare phase measurements, at a range of 500 Hz- 5 KHz.

4- Test for the phase at a glycerin level of 0%, or distilled water. This is done for five trials. Between each trial the probe and wires are moved, to simulate the placement of another sample of blood.

5- Step 3 is repeated for all ten levels of glycerin.

The protocol above summarizes the experiments implemented in order to test the efficacy of the probe in mediums of different viscosity levels. After three different prototypes, consistent results were successfully produced with these methods. The final prototype was able to overcome the fragility of the first prototype and the cost and delamination issues of the second. The completion of these tests represents the functioning of the probes in a static, or constant, environment. To ensure that the probes will also work in detecting time varying viscosity changes, dynamic testing is also necessary.

C. Dynamic Testing

The previous experiments can only tell us the success of the probe in a static solution. For that reason, a dynamic environment needed to be set up in order to test if it would be just as successful in a changing medium. In order to do just that, an environmental heat chamber was used (Sun Systems Environmental Test Temperature Chamber). Using a program via Lab View, a vacutainer of glycerin solution was placed in the chamber and gradually heated to various temperatures between room temperature (25°C) and 100° C. At different points during this process, the solution was quickly taken out and tested for phase to simulate the time varying change in viscosity of whole blood, outside the human body. Probe 2 was tested at room temperature, 40°C, 80° C, and 90° C and is shown in the results section. Temperature and time have a linear relationship and can therefore be referred to interchangeably in this association. This set of experiments should ideally represent the clotting process of blood, with changes in viscosity being detected as the clot forms.

CHAPTER 3

Results and Discussion

A. Results

After several different trials and tests it was found that the phase shift frequency differs for each viscosity level and was relatively consistent, making it the most appropriately sensitive method for later monitoring whole blood coagulation. Frequencies ranging from 100 Hz to 50 MHz were tested for each glycerin solution level. It was found that at the higher frequencies, results were extremely inconsistent as far as repeatability. Phase shift values for each level of glycerin solution were later compared to known viscosity values for their respective glycerin percentage, in the form of a calibration curve. The dynamic tests, or temperature experiments, revealed an inverse relationship between time and phase shift frequency.

The following is a partial chart of glycerin viscosities in terms of both temperature and solution percentage (Table 4). As stated earlier, room temperature values, or 25° C, were interpolated from this chart. This source also did not give the

viscosity for 25%; it had to be interpolated using the surrounding values, producing a viscosity of 1.95 cP. Although glycerin levels between 0 and 100 % were tested during experimentation, it is important to note that whole blood viscosities tend to only fall in the 25 to 50% glycerin range (Kanaris et al., 2010).

Table 4. Chart of Glycerin level viscosities in cP ("Glycerine- A Review," 1990).

Glyc. % Wt.	Temperature (°C)					
	0	10	20	30	40	50
0*	1.792	1.308	1.005	0.8007	0.6560	0.5494
10	2.44	1.74	1.31	1.03	0.826	0.680
20	3.44	2.41	1.76	1.35	1.07	0.879
30	5.14	3.49	2.50	1.87	1.46	1.16
40	8.25	5.37	3.72	2.72	2.07	1.62
50	14.6	9.01	6.00	4.21	3.10	2.37
60	29.9	17.4	10.8	7.19	5.08	3.76
65	45.7	25.3	15.2	9.85	6.80	4.89
67	55.5	29.9	17.7	11.3	7.73	5.50
70	76	38.8	22.5	14.1	9.40	6.61
75	132	65.2	35.5	21.2	13.6	9.25
80	255	116	60.1	33.9	20.8	13.6
85	540	223	109	58	33.5	21.2
90	1310	498	219	109	60.0	35.5
91	1590	592	259	127	68.1	39.8
92	1950	729	310	147	78.3	44.8
93	2400	860	367	172	89	51.5
94	2930	1040	437	202	105	58.4
95	3690	1270	523	237	121	67.0
96	4600	1580	624	281	142	77.8
97	5770	1950	765	340	166	88.9
98	7370	2460	939	409	196	104
99	9420	3090	1150	500	235	122
100	12070	3900	1410	612	284	142

A series of formulas were investigated in order to produce a strong relationship of viscosity to the phase shifts. Three different manipulations were attempted and the graph with the strongest correlation was eventually chosen to represent the calibration curve. The first method was a direct plot of the phase shift frequency to the

corresponding viscosity value (Figure 23 and 24). Regression lines of the rational form $F=(a+b*x)/(1+c*x)$ were created.

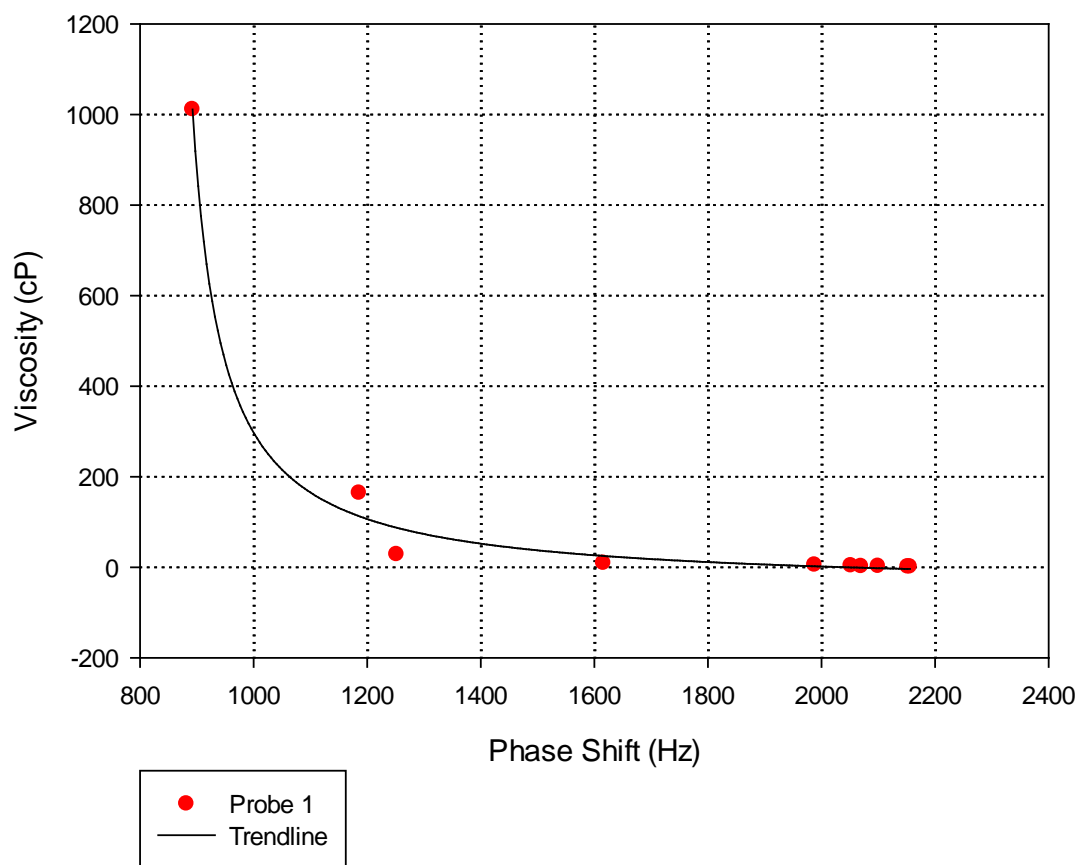


Figure 23. Method 1: Phase Shift Frequency v. Viscosity of Probe 1.

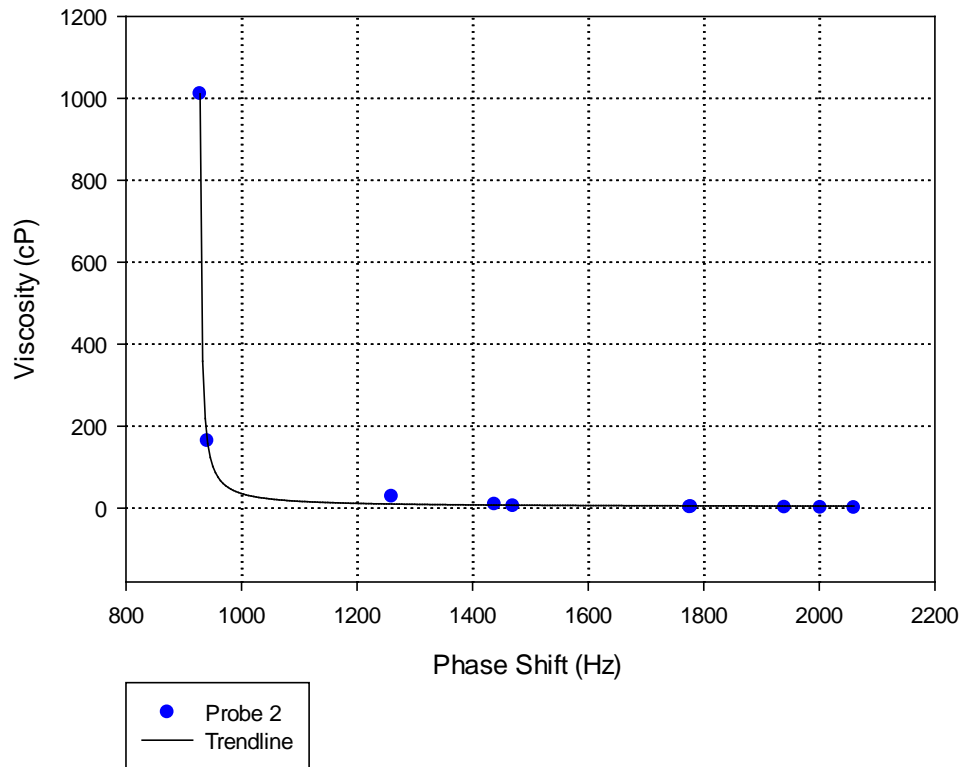


Figure 24. Method 1: Phase Shift Frequency v. Viscosity of Probe 2.

The second method was of normalized phase shift frequency values to viscosity. The normalization was done by dividing the phase shift frequency by the physical property of the probe, or the peak impedance phase shift frequency; we called this normalized value alpha (α).

$$\text{Equation 2: } \alpha = \text{Phase Frequency} / \text{Impedance Frequency}$$

This is graphed for the two final probes in Figures 25 and 26, also with an equation of rational form.

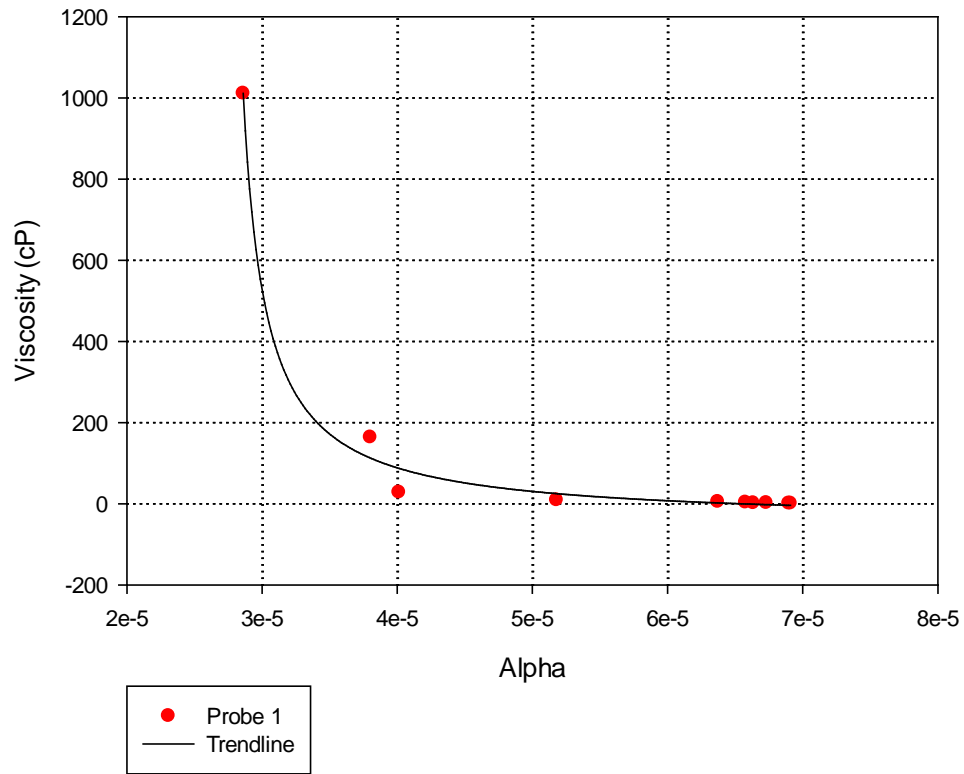


Figure 25. Method 2: Normalized Phase (Alpha) v. Viscosity for Probe 1.

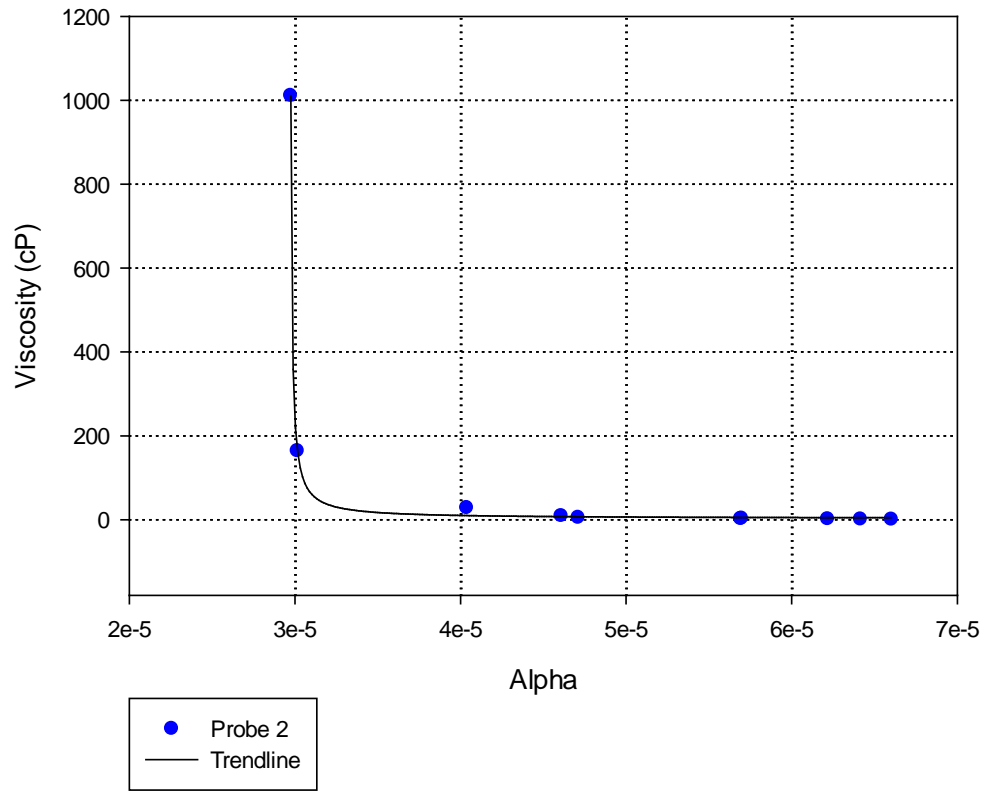


Figure 26. Method 2: Normalized Phase (Alpha) v. Viscosity for Probe 2.

Finally, a difference equation versus viscosity was attempted. The formula used to produce the graphs seen below is:

$$\text{Equation 3: } \eta = 'Abs(PhaseFrequency-Impedance)/(Phase Frequency)'$$

This final manipulation uses a simple formula that takes into account a property of the individual probe, or impedance in air, as well as the measured phase shift frequency. This value is represented by the symbol η . The formula was then graphed versus viscosity, as seen in Figure 27 and 28.

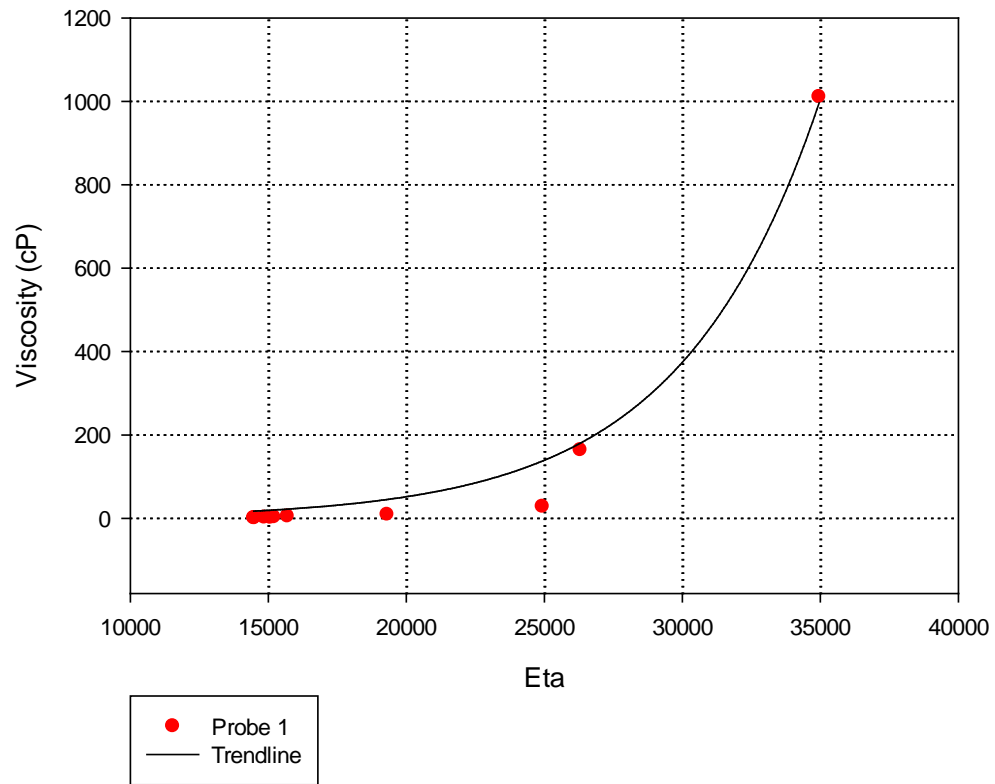


Figure 27. Method 3: Eta v. Viscosity for Probe 1.

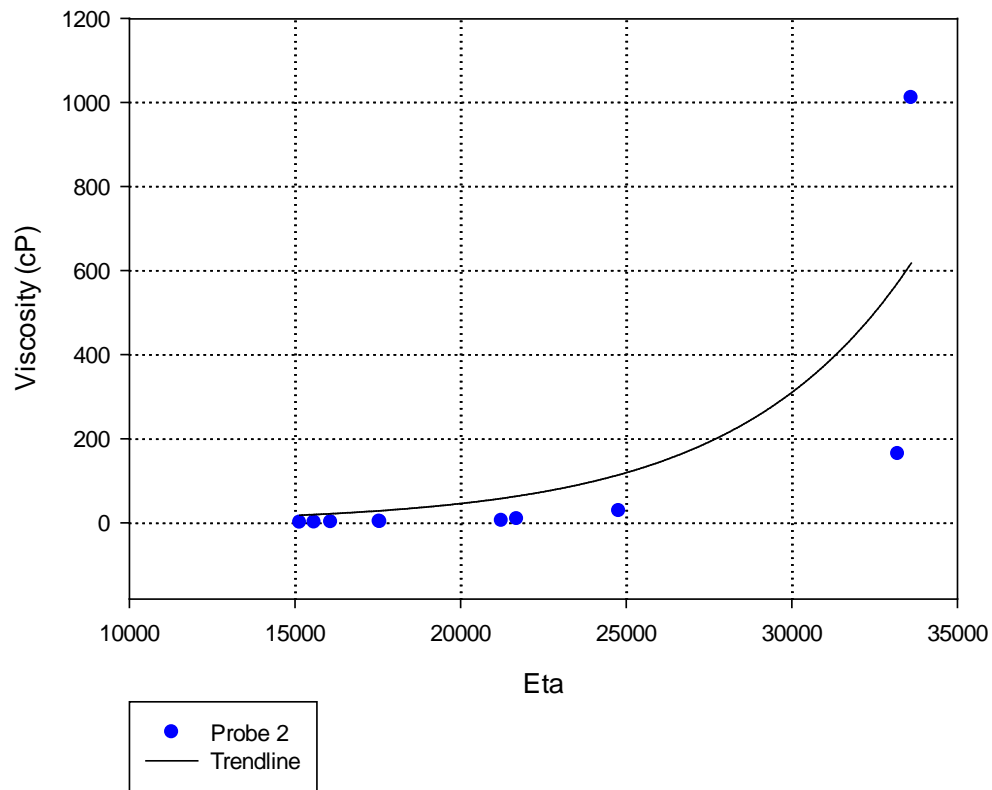


Figure 28. Method 3: Eta v. Viscosity for Probe 2.

Of the three methods, the strongest correlation was produced by the rational equations of method 2, the normalized phase curves. Statistical values specific to each probe are discussed below.

I. Probe I

Probe I had an average percent error of 0.95%. This percent error was calculated by dividing standard deviation by the average, from a set of five trials per solution percentage. The highest level of variability in repeatability fell between 50 and

75% glycerin solution. Once α was graphed versus viscosity the resulting correlation produced a rational equation. Using non-linear regression, the equation produced was:

$$\text{Equation 4: } \text{Viscosity} = (-110.36 + 1.687E6 * \alpha) / (1 - 3.71E4 * \alpha)$$

Figure 25, shows a graph of Probe 1 results, while Table 5 depicts the values, averages, and calculated statistics. The correlation coefficient, or R, was 0.99.

Table 5. Results of Probe 1.

Gly (%)	Visc (cp)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average Phase (KHz)	Standard Deviation	% Error
0	0.9	2.040	2.040	2.060	2.070	2.090	2.060	.02121	1.030%
10	1.17	1.980	1.990	2.010	2.010	2.020	2.002	.01643	0.821%
25	1.95	1.930	1.940	1.950	1.940	1.940	1.940	.00707	0.364%
30	2.19	1.750	1.780	1.790	1.770	1.790	1.776	.16733	0.942%
40	3.22	1.780	1.780	1.780	1.780	1.770	1.778	.00447	0.252%
50	5.11	1.450	1.400	1.490	1.500	1.510	1.470	.04527	3.080%
60	9	1.450	1.440	1.440	1.420	1.440	1.438	.01095	0.762%
75	28.35	1.200	1.270	1.280	1.250	1.300	1.260	.03807	3.022%
90	164	.896	.901	.971	.977	.960	9.410	.03931	4.178%
100	1011	.922	.933	.927	.933	.927	.928	.00466	0.503%

II. Probe II

Similar results were produced with the second probe. This probe had a percent error of 1.5% and a correlation coefficient of 0.99. Probe 2's phase shifts were of a similar frequency range to those of probe 1. Its calibration curve equation was also of a

rational time, but with different coefficients. Figure 26, is that of Probe 2's graph and Table 6 is of its statistics, similarly to those of Probe 1.

$$\text{Equation 5: } \text{Viscosity} = (0.158 - 9.376E4 * \alpha) / (1 - 3.369E4 * \alpha)$$

Table 6. Results of Probe 2.

Gly (%)	Visc (cP)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average Phase (KHz)	Standard Deviation	% Error
0	0.9	2.150	2.140	2.150	2.150	2.170	2.152	.01095	0.509%
10	1.17	2.140	2.150	2.170	2.170	2.150	2.156	.01341	0.622%
25	1.95	2.060	2.060	2.070	2.070	2.090	2.070	.01224	0.592%
30	2.19	2.090	2.090	2.100	2.100	2.120	2.100	.01224	0.583%
40	3.22	2.040	2.040	2.040	2.070	2.070	2.052	.01643	0.801%
50	5.11	2.010	2.010	1.980	1.980	1.960	1.988	.02167	1.091%
60	9	1.590	1.600	1.630	1.630	1.630	1.616	.01949	1.206%
75	28.35	1.290	1.270	1.240	1.240	1.220	1.252	.02774	2.216%
90	164	1.200	1.190	1.190	1.170	1.180	1.186	.01140	0.961%
100	1011	.896	.901	.896	.890	.880	.893	.08049	0.902%

III. Temperature Experiment Results

As blood coagulates, it gets more viscous. The prediction, based on our calibration curves, is that phase shift frequencies will decrease as blood clots, as they did with higher, more viscous percentages of glycerin solution. Temperature experiments were done to make a valid connection between the preliminary results and the prospect of eventually being able to monitor changes in whole blood. A static measurement at a specific viscosity does not tell us much in terms of real-time monitoring. Figure 29 shows the various phase shifts produced at different times, of a 30% solution. For better visualization, the derivative is also graphed in Figure 30. It is

clear that frequencies of phase shifts are indeed slightly lower with the passing of time.

It can be inferred that as viscosity is changing with time, frequency will also decrease.

Figure 31 shows this relationship in decreasing linear plot, indicating a direct relationship between viscosity and phase in a dynamic environment.

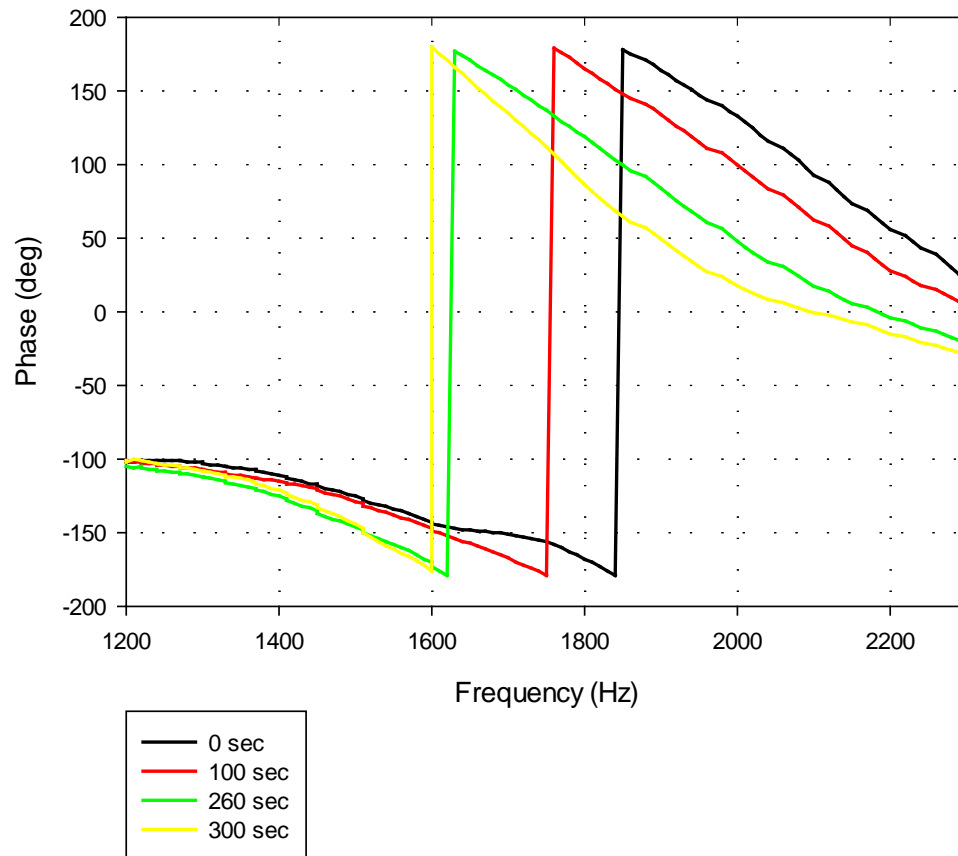


Figure 29. Dynamic Results: 30% Glycerin Solution.

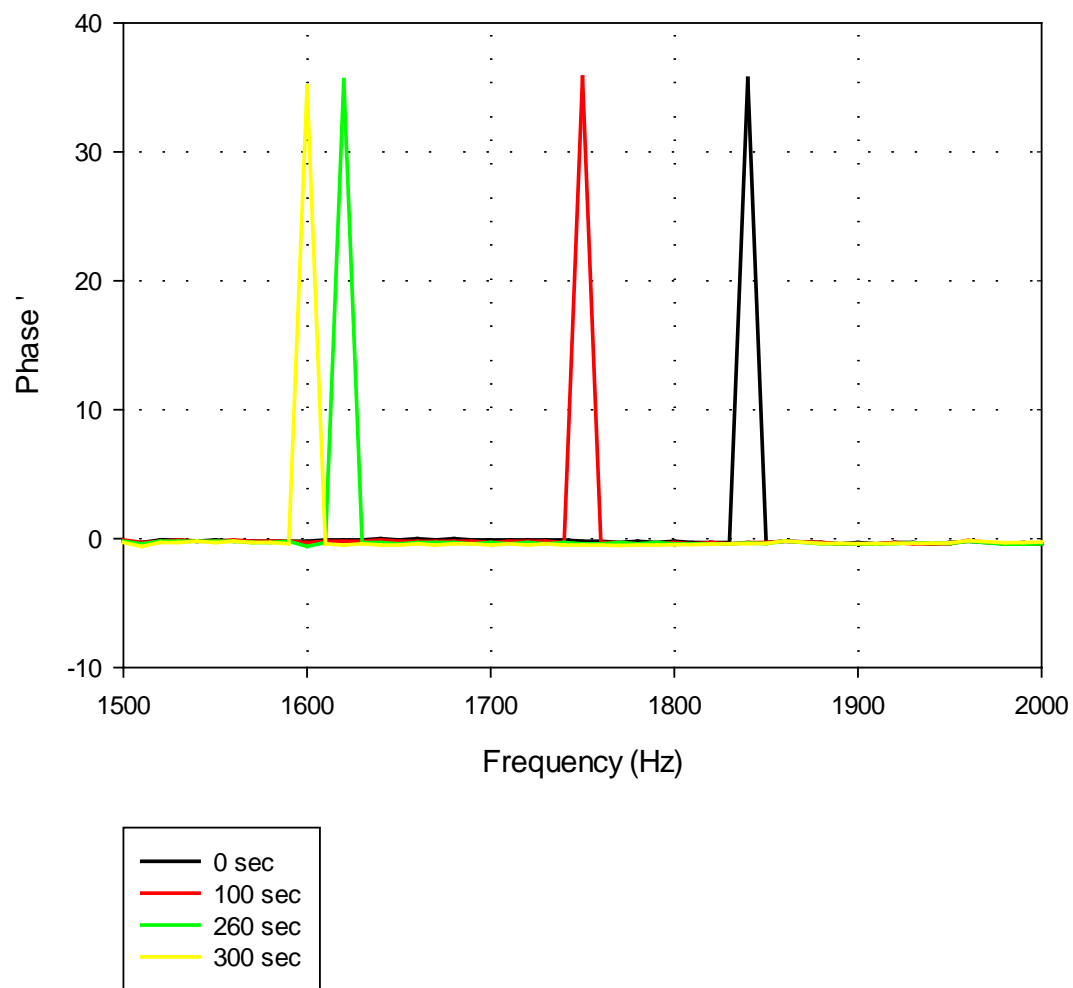


Figure 30. Derivative of Phase shifts from Figure 30.

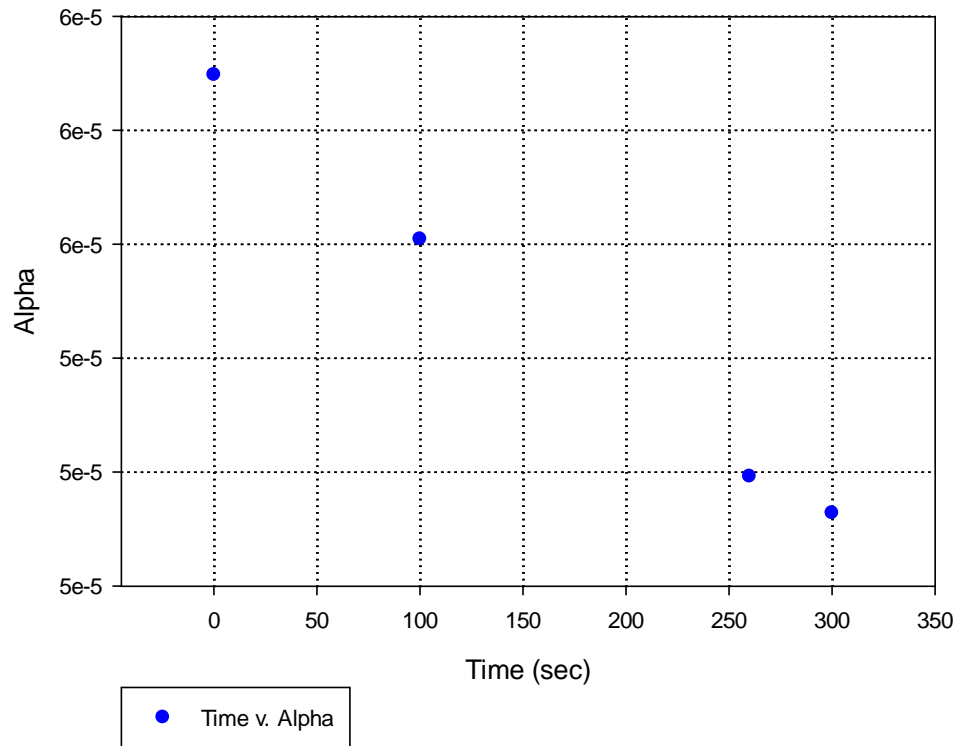


Figure 31. Time v. Alpha graph. As viscosity is changing with time, the frequency shift decreases.

B. Discussion

The final results, of five trial sets, have shown that the probes do not produce the same results when tested in the same solution. This was most likely due to the fact that the entire probe was manmade, with several variables. However, the same probe does have a limited and acceptable level of variability, with both presented probes having an average percent of error of less than 2%. In terms of repeatability, or various trials with the same probe, the results are promising. However, the replication from probe to probe was exceedingly different. For this reason, the calibration curves created for each tested probe are necessary. Both final probes had a correlation coefficient greater than 0.95, which is more than acceptable for a regression line. It is also notable that there were concrete differences between glycerin solutions of only 5% viscosity changes, or more specifically less than 0.5 cP differences. This observation reveals the sensitivity and promise of this system.

The probes need not be identical to each other, but it is required that they have the ability to reproduce the same phase shift frequency when repeatedly tested. The low percent error, high coefficient of determination, and ability to distinguish between slight changes in viscosity suggests that these probes are also capable of performing in whole blood. The temperature experiments reveal similar results; the probes are also capable of doing so in a dynamic environment.

C. Further Research

Two important phases were completed on the path to successfully monitoring whole blood. Probe properties, frequency ranges and measurement method were characterized. Not only do we know what these unimorphs are capable of measuring, but we also have an idea of their sensitivity to viscosity changes. The temperature experiments have also revealed that dynamic measurements are indeed possible with this prototype. The next step of this project would be to test the probes in whole blood and compare results. Ideally, the phase shifts at different frequencies should be replicated with the gradual clotting of a sample of blood. For example, at initiation of the clot, a shift at approximately 2.1 kHz could be expected, while a complete fibrin clot would produce a phase shift at a lower frequency, similar to that of 50% or 60% glycerin solution. The amount of time a phase shift of the lower frequency value takes to occur could be an indication of the patient's coagulation rate. Obviously, the rate of clotting (change in phase) and strength of the clot (phase shift frequency) will differ from patient to patient, due to variations of hematocrit, medication, age, type of disease, and other significant factors.

Aside from replication issues, several challenges will be faced once whole blood is sampled for testing. Whole blood's diverse composition and varying densities may cause some problems with the probe when it comes time to make viscosity measurements. Red blood cell count, or hematocrit, makes up approximately 50% of a

whole blood sample. The viscosity of plasma is only up to 1.8 times that of water, while the viscosity of whole blood is up to 5.5 times that of water. This is due to the existence of particles such as red blood cells and platelets, which are lacking in plasma (Rhoades and Bell, 2012). Red blood cells are very flexible and are constantly changing shape. This implies that when a sample of whole blood is placed in the vacutainer for testing, the displacement of the fluid is going to be constantly affected by the movement and shape of these particles. Smaller samples are also possible with a simple redesign in the length of the probe; the longer the unimorphs, the less blood sample will be required to fill the vacutainer to its appropriate height.

Another challenge that we may face in the next level of testing involves the conductive properties of whole blood. The electrical conductivity of blood is affected by hematocrit, RBC characteristics, protein concentration and the relative step in clotting at the time (Hirsch et al., 2011). The sample's electrical conductivity may or may not alter the piezoelectrics and therefore the output signal given by the probe's sensor. Numerous tests in healthy plasma, RBCs, and whole blood will need to be completed in order to verify and take into account all of the projected challenges.

D. Conclusion

Several successful steps have been met and completed on the path to creating a piezoelectric probe capable of monitoring blood coagulation. Materials have been selected, approximate shape and size chosen, and appropriate frequency ranges have been confirmed. The final prototype has met various constraint requirements, such as size, sample volume, and durability. The probe's design has proven to be small enough to fit into a 3 mL vacutainer, require only 1.5 mL of liquid, and sturdy via gripping of external wiring with shrink wrap. Probes have also been tested in both static and dynamic conditions and produced acceptable and consistent results.

Both final probes demonstrated an average percent error of less than 2% when it came to repeatability. They also had regression correlation coefficients greater than 0.95- corresponding to a strong trend of frequency data with changing viscosities. By observing significant differences between 25% and 30% glycerin solutions at room temperature (or 0.5 cP difference) we can also infer that this method of measuring viscoelasticity is exceptionally sensitive to even small changes in viscosity. Temperature experiments also revealed that the probes are just of capable at monitoring viscosity in a dynamic environment. A disadvantage to fabricating the probes in house is the lack of replication from probe to probe. This difference was overcome by creating calibration curves for each individual probe. A calibration equation allows the experimenter to

calculate viscosity based on α , or the sample's viscoelastic strength using only the produced frequency of a phase shift.

In addition to the calibration curves and temperature experiments, the following steps need to be taken in order for the device to be ready for field-testing. Ideally, the next phase is early clinical testing in bovine red blood cells (RBCs), plasma, and whole blood. Bovine samples are commonly used and therefore readily available. As previously stated, RBCs and plasma will function differently due to their distinctive characteristics and viscosities. The final prototype should also be able to produce accurate results despite variability in patients and patients' conditions.

Piezoelectric sensors have been successful in a wide range of applications, such as in the monitoring of the structural health of buildings, which suggests their capability to provide accurate measurements (Giurgiutiu, 2008). In order to verify success, however, the probes will need to react and function similarly in whole blood. By overcoming the TEG's issues of disturbing the clot during monitoring, our technology has the potential to become a leading bedside monitor of blood coagulation in the OR. As a POC technology, it is expected that this system not only be simple to use, but also have a short TAT and the ability to present the output in real-time. The advantages of the proposed method over the current conventional ones are that the system is small and disposable. Most importantly, it has the potential to be very cost effective, as opposed to the current methods which range in the tens of thousands of dollars. By continuing experimentation on time varying viscosities and clotting whole blood, as well

as manufacturing identical probes, it may be possible to successfully produce an inexpensive, real-time POC device to monitor whole blood coagulation.

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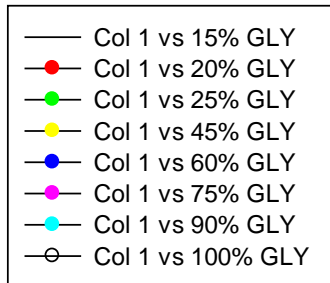
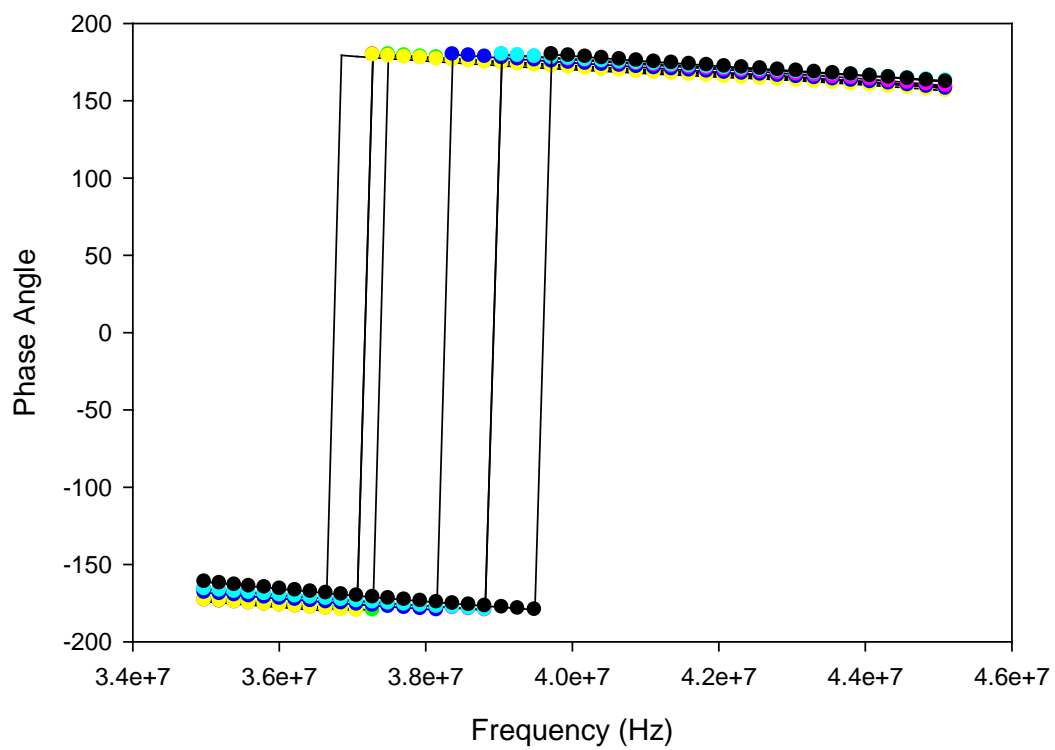
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APPENDIX

Preliminary Phase Testing:

Frequency vs. Phase Shift



Preliminary Testing continued.

Frequency vs. 1st dydx of Phase Angle WRT Frequency

